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European corn borer: components of plant resistance and concentrations of 1,4-benzoxazin-3-ones in corn

John Frank Robinson
Iowa State University

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Zoology and Entomology
Major: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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For the Graduate College

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INTRODUCTION

The relationship between concentrations of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and leaf feeding resistance to first generation European corn borer, Ostrinia nubilalis (Hubner), has been well established (Klun et al.,1967) However, in two of the eleven inbred lines of corn, Zea mays L., studied there is a higher concentration of DIMBOA than their relative leaf feeding ratings would indicate (Klun et al., 1970). This suggests the possibility that other compounds, possibly homologs of DIMBOA, are exerting an effect on the resistance mechanism.

This research was undertaken to further elucidate the nature of first generation European corn borer host plant resistance in terms of larval mortality, establishment and movement off the plant and the relationship between these biological phenomena and the concentrations of five 1,4-benzoxazin-3-ones found in selected inbred lines of dent corn.

LITERATURE REVIEW

Host plant resistance research is a study of the pest-host plant relationship in an attempt to discover heritable plant characteristics, which can be utilized in a practical agricultural system to lessen the economic losses incurred from a phytophagous pest. Resistant host plants characteristically have less pest damage or fewer pest numbers than other host plants grown under the same environmental conditions (Painter, 1951). The level of host plant resistance in a specific variety is relative and is described in terms of other and usually more damage-prone (susceptible) varieties (Maxwell et al., 1972).

The nature of host plant resistance has been divided into three components. These components are descriptive terms which serve to characterize the type of resistance found in a particular plant. A resistant host plant may possess any one or any combination of the three components. The three components of insect-host plant resistance are: 1) antibiosis - an adverse effect on the insect's biology when it feeds on a resistant host plant which may contain a toxic material or lack some essential nutrient, 2) non-preference - an adverse effect on the insect's behavior when it attempts to utilize a resistant host plant for food, oviposition, shelter or a combination of the three, 3)

tolerance - a resistant plant which yields well despite supporting insect numbers which would severely damage a susceptible plant (Painter, 1951, 1958; Beck, 1965).

A more thorough coverage of the terminology, concepts, examples and economics of insect-host plant resistance and definitions of the behavioral aspects of the plant-insect relationship has been presented in the following articles: Beck (1965), Dethier et al. (1960), Dethier (1970), Horber (1972), Luginbill (1969), Maxwell et al. (1972), National Academy of Sciences (1969) and Painter (1951, 1958, 1968).

Some knowledge of the insect- host plant relationship and the presence of resistance in the host species must be determined before a study of the chemical nature of the resistance is undertaken. The insect and the host plant are dynamic systems and the nature of their relationship is often complex. Host plant resistance defined in terms of this relationship can be described as a "lack of fit" between the biological characteristics of the two systems (Beck, 1965).

The relationship between European corn borer larvae and the corn plant has been described as simple (Beck, 1965). European corn borer larvae, although polypahgous, usually depend upon the host plant selected by the adult female to furnish the requisites needed for survival.

First generation European corn borer larvae feed and establish on corn plants at the whorl stage of plant development. These larvae feed upon the loosely rolled leaves of the whorl in close association with an area of moisture formed by water entrapped within the whorl. The larvae feed and maintain their relationship with the moist area, and as the leaves extend, elongated feeding scars are formed on susceptible plants and small, round feeding scars are formed on highly resistant plants (Dicke and Penney, 1954).

Field evaluations of first generation European corn borer host plant resistance are based on a nine-class rating scale. The amount of leaf feeding damage is visually assessed and the plant is assigned a numerical value on the rating scale (Guthrie et al., 1960). A rating of 1-2 is considered highly resistant, a rating of 3-4 is considered resistant, a rating of 5-6 is considered intermediate in resistance, and a rating of 7-9 is considered highly susceptible (Guthrie and Dicke, 1972).

Beck and co-workers at the University of Wisconsin initiated a study on the biochemical basis of first generation European corn borer resistance. They found European corn borer larval growth inhibitors in two chemical fractions of the corn plant. These fractions contained at least three inhibitory materials which were labeled resistance factor (RF) A, RFB and RFC (Beck and Stauffer, 1957; Beck, 1957).

RFA was isolated (Loomis et al., 1957), synthesized and identified as 6-methoxybenzoxazolinone (MBOA) (Smissman, 1957a,b). RFC was identified as 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Beck, 1965) although no experimental proof of this identification was given. RFB was never isolated or identified (Beck, 1965). The corn plant concentration of MBOA was related to: plant maturity, tissue from which it was extracted and the variety used as a source (Beck and Stauffer, 1957; Loomis et al., 1957; Beck, 1957). These findings were later confirmed by Klun and Robinson (1969). MBOA was found not only to inhibit larval growth but also to act as a feeding deterrent. It was concluded that these two effects of MBOA contributed to the field expression of corn plant resistance to European corn borer larvae (Beck, 1957, 1965).

MBOA was isolated (Virtanen et al., 1956) and identified (Hietala and Wahlroos, 1956) from crushed maize and wheat plants. Wahlroos and Virtanen (1959a) found that MBOA did not exist in intact maize and wheat plants but was formed from a glucosidic precursor when the plants were crushed and the extract heated. These findings led Virtanen (1961) to hypothesize that MBOA could not be the toxic factor involved in European corn borer host plant resistance as was indicated by Beck. Wahlroos and Virtanen (1964) proved that MBOA did not exist in fresh corn plant tissues and that the aglucone

precursor (DIMBOA) was present in considerable amounts. They suggested that the aglucone was the resistance factor found in corn plant tissues.

Klun and Brindley (1966) found that a high concentration of MBOA was correlated with a low resistance rating. From this and subsequent bioassay tests, a partial biochemical explanation of European corn borer leaf feeding resistance has been developed (Klun et al., 1967; Klun, 1970). A glucoside, 2,0-glucosyl-4-hydroxy-1,4-benzoxazin-3-one, exists in uninjured corn tissue (Wahlroos and Virtanen, 1959a; Hofman and Hofmanova, 1969). When corn plant tissue is damaged (larval feeding), the glucoside is hydrolyzed by a glucosidase to the aglucone, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Wahlroos and Virtanen, 1959b). DIMBOA was shown to be biologically active against European corn borer larvae (Klun et al., 1967). DIMBOA slowly decomposes to MBOA (Klun et al., 1967). MBOA is chemically stable and has little or no biological activity against European corn borer larvae (Klun and Brindley, 1966; Klun et al., 1967).

The genetic nature of DIMBOA concentrations and resistance to European corn borer was studied in a diallel set of eleven corn inbreds (Klun et al., 1970). Additive and/or additive X additive epistatic effects were of primary importance in both the resistance rating system and

DIMBOA chemical analysis. They concluded that the utilization of a DIMBOA chemical analysis in a resistance screening program would be equally as effective as the visual rating system currently in use.

DIMBOA has also been shown to inhibit Helminthosporium turcicum spore germination (Coutre et al., 1971), and to be associated with resistance in cereal grasses to fungi (BeMiller and Pappelis, 1965; Elnaghy and Linko, 1962; Molot and Anglade, 1968).

The 1,4-benzoxazin-3-ones isolated from corn are: 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) (Tipton et al., 1967), 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Wahlroos and Virtanen, 1959a,b), 2,4-dihydroxy-6,7-dimethoxy-1,4-benzoxazin-3-one (DIM_2BOA) (Klun et al., 1970), 2-hydroxy-7-methody-1,4-benzoxazin-3-one (HMBOA) (Tipton et al., 1967; Gahagan and Mumma, 1967), 2-hydroxy-1,4-benzoxazin-3-one (HBOA) (Hofman and Hofmanova, 1969). The biosynthesis of HBOA, HMBOA, DIBOA and DIMBOA has been discussed by Tipton et al. (1972). Anthranilic acid was found to be a precursor in benzoxazinone biosynthesis. The results from a feeding experiment suggested that HBOA was converted either to DIBOA or HMBOA, with HMBOA being converted to DIMBOA. The biosynthesis of DIM_2BOA is presently unknown.

Plant concentrations of DIMBOA are usually determined by

chemical analysis for MBOA. MBOA is a relatively stable chemical entity and its concentration is stoichiometrically related to DIMBOA concentration. It should be remembered that bioassay tests with European corn borer larvae have shown that DIMBOA is biologically active and that MBOA is not (Klun et al., 1967).

METHODS AND MATERIALS

Greenhouse and Field Tests

Selected inbred lines of dent corn with different levels of first generation European corn borer resistance were studied in greenhouse tests (1969 and 1970) and in a field test (1971) to determine if larval migration (movement) off the plant was a component of European corn borer first-generation host-plant resistance. Larval migration was measured by trapping and then counting the larvae which moved off the plant. A technique of this type was used previously with greenhouse grown corn (Guthrie and Huggans, 1967; Huggans, 1968). Table 1 shows the numerical and descriptive field resistance ratings for the inbreds used in these tests.

Table 1. First generation European corn borer numerical and descriptive field resistance ratings for inbreds WF9, Oh43, CI31A, B52, W22 and R101[a]

Inbred	Range Numerical Rating	Descriptive Rating
WF9	7-9	Highly susceptible
R101	7-9	Highly susceptible
W22	5-6	Intermediate in resistance
B52	5-6	Intermediate in resistance
Oh43	3-4	Resistant
CI31A	1-2	Highly resistant

[a]Data from Guthrie and Dicke (1972).

Greenhouse tests

A greenhouse was constructed inside the shop area of the European corn borer laboratory at Ankeny, Iowa. A wooden framework, 9.2 m (length) x 7.5 m (width) x 2.5 m (height), was constructed and then the inside and outside surfaces were covered with clear plastic sheeting. Artificial light was supplied by twelve 2.5 m (length) fluorescent lights. The lights were programmed on a 16 hour light: 8 hour dark cycle. Relative humidity (66%-92%) was obtained by spraying the walls and floor with water, at least twice daily. The temperature inside the greenhouse, ca. 1 m above the floor, ranged from 25°C-30°C.

Field-grown corn plants, ca. 91 cm in extended leaf height were transplanted into 25.4 cm (dia) x 25.4 cm (height) clay pots. The plants were transferred to the greenhouse and a trap was placed around each plant. The trap consisted of two, 61 cm x 121.8 cm x 30.5 cm (height) tables. The center of one long side of each table was notched. Two tables were fitted around the stalk of each plant to form a 121.8 cm x 121.8 cm trapping surface beneath each plant. The plant extended through the center of this surface and was positioned so no leaves extended over the edge or touched the trap surface. The table surface beneath each plant was then covered with freezer paper and coated with Stikem ®. To trap larvae that might move down the corn stalk, a ring of

Stikem ® was placed around the plant at its junction with the trap surface. Thirty first-instar European corn borer larvae were placed in the whorl of each plant.

Two tests were conducted in 1969 with inbreds WF9, Oh43 and CI31A, and three tests were run in 1970 with inbreds WF9, OH43, CI31A, B52, W22 and R101. Each test was designed as a completely random design. At the end of four days, each plant was dissected. Surviving larvae were counted and weighted to obtain the number of larvae established/plant and the average weight/larva/plant. The number of larvae found on the trap surface beneath each plant was recorded daily in tests 1, 2 and 3 and at the end of 4 days in tests 4 and 5. Larval mortality on each plant was calculated using the equation: initial population - number established - number trapped = mortality on the plant. total mortality/plant is reflected in the number of larvae established (total mortality = number trapped + number dead on plant or total mortality = initial population - number established). In each test, extended leaf heights were taken and tassel bud ratios (Luckmann and Decker, 1952) were calculated. A total of 134 individual plants were evaluated in this manner.

After the plants were dissected in 1970 (tests 3, 4 and 5), the whorl leaf tissue was bulked by inbred within a test and frozen. These whorl tissue samples (3 samples/inbred) were subsequently analyzed for HBOA, HMBOA, DIBOA, DIMBOA and DIM_2BOA content.

Field test

The collection of wind-borne debris on the trap surface and the possibility that migrating larvae might be blown outside the trap surface indicated that the greenhouse traps were unsuitable for the field study. Therefore, field traps were designed as topless boxes, 121.8 cm x 121.8 cm x 61-cm high, and were constructed from 1/4" plywood around plants ca. 84 cm in extended leaf height. The traps were positioned so that the plant extended through the center of the base and no leaves touched the trap surface. The inside of the trap surface was lined with freezer paper and then coated with Stikem®. A ring of Stikem® was also placed around the plant at its junction with the trap surface. Thirty first-instar European corn borer larvae were placed in the whorl of each plant. At the end of 4 days, the plants were dissected and larval migration/plant, larval establishment/plant, and larval mortality/plant were assessed as before.

The field test was designed as a randomized complete block. A block consisted of one plant of each variety and the test was blocked over three time periods. The inbred lines included in this test were: WF9, Oh43, CI31A, B52, W22 and R101.

Two aggregate samples of whorl tissue of each inbred (5 plants/aggregate sample) were cut and frozen for chemical analysis. The first aggregate sample was taken when the

plants were 71 cm - 81 cm in extended leaf height and the second aggregate sample was taken when the plants were 91 cm - 101 cm in extended leaf height. Tassel bud ratios were not taken.

Chemical Analysis

Concentrations of HBOA, HMBOA, DIBOA, DIMBOA and DIM_2BOA were determined by chemical analysis of whorl tissue (collected from six inbred lines of corn). The cyclic hydroxamic acids, DIBOA, DIMBOA and DIM_2BOA, are chemically laible and are stoichometrically related to their chemically stable degradation products, benzoxazolinone (BOA), 6-methoxybenzoxazolinone (MBOA) and 6,7-dimethoxybenzoxazolinone (M_2BOA), respectively. The cyclic hydroxamic acid concentrations were determined by chemical analyses for the benzoxazolinones. The lactams HBOA and HMBOA are chemically stable and were analyzed for directly.

Several methods of chemical analysis have been reported to determine varietal differences of whorl concentrations of MBOA (Beck et al., 1957; Klun and Brindley, 1966; Bowman et al., 1968; Klun and Robinson, 1969; Klun et al., 1970). The isotope dilution analysis developed by Klun and Robinson (1969) for BOA and MBOA and the later extension of this method for M_2BOA (Klun et al., 1970) was modified for these analyses. No labeled HMBOA was available so a spectrophotometric chemical

analysis was developed for this material. The ^{14}C-labeled, BOA (specific activity (SA) 60.76 µc/m mol), MBOA (SA 147.88 µc/m mol), and M_2BOA (SA 60.06 µc/m mol), were supplied by Dr. J. A. Klun of the Ankeny Laboratory. Synthesis of these compounds has been described previously (Klun and Brindley, 1966; Klun and Robinson, 1969; Klun et al., 1970). The ^{14}C-labeled HBOA (SA 21.26 µc/m mol) was synthesized (Tipton et al., 1973) and supplied by Dr. C. L. Tipton of Iowa State University.

Corn plant whorl tissue was collected, frozen, dried (50 °C) and then ground. Two hundred and fifty mg of the tissue was placed in a 2.7 cm dia x 5.8 cm height (25 ml vol) screw-capped vial. Fifty µℓ (95% ethyl alcohol soln.) of ^{14}C-labeled, BOA (20,295.33 dpm), MBOA (24,566.1 dpm), M_2BOA (26, 485.36 dpm) and one hundred µℓ (95% ethyl alcohol soln.) ^{14}C-labeled HBOA (13,215.6 dpm) were injected into the vial. An aqueous extract was prepared by adding 20 ml of boiling water and agitating for one minute on a Vortex mixer. The aqueous extract was filtered, 6 ml of the filtrate acidified to pH 1-2 with concentrated HCl acid, and extracted with 12 ml of diethyl ether. Six ml of the ether extract was removed and evaporated under a lab hood (ca. 20 °C). The ether soluble residue was then taken up in 400 µℓ benzene-ethyl acetate (1:1 v/v). One hundred and fifty µℓ of this solution was spotted as a single spot on a 20 cm x 20 cm glass plate

covered with a thin layer of silica gel GF_{254} (Brinkman Instruments, Westbury, New York). The thin layer chromatography (TLC) plate was developed in two dimensions to resolve HBOA, HMBOA, BOA, MBOA and M_2BOA. The TLC plate was developed in one dimension with chloroform-ethylacetate-cyclohexane (4:4:2 v/v) and then in the second dimension with cyclohexane-isobutanol (85:15 v/v). These solvent systems were developed previously by Klun and Brindley (1966). Table 2 shows the Rf values of the five compounds in the two solvent systems.

The identity of the isolated compounds was confirmed by removing the materials from the TLC plate and co-chromatographing the plant-isolated materials with standard synthetic samples, the ultra-violet (UV) absorbance spectra (in 95% ethyl alcohol) of the isolated compounds were identical to the UV absorbance spectra of synthetic standards. A

Table 2. Rf values obtained from 2-dimensional TLC of HBOA, HMBOA, BOA, MBOA and M_2BOA

Solvent System	HBOA	HMBOA	BOA	MBOA	M_2BOA
Chloroform-ethyl acetate-cyclohexane (4:4:2 v/v)	0.34	0.26	0.69	0.58	0.56
Cyclohexane-isobutanol (85:15 v/v)	0.46	0.38	0.53	0.49	0.36

Beckman Model DB Spectrophotometer® was used to prepare the standard curve for each of the compounds. The solutions of the compounds conformed to Beer's law for an ideal solution over the range of concentrations tested. The standard curve slope for each compound is shown in Table 3.

The TLC chromatograms were viewed under short wage UV light to visualize the compounds of interest. The silica gel, corresponding to the Rf of each compound, was removed from the plate and was transferred to a disposable Pasteur pipette plugged with 95% ethyl alcohol washed glass wool. The compound

Table 3. Standard curve slopes for HBOA, HMBOA, BOA, MBOA and M_2BOA

Compound	Wave length (mμ) standard curve developed	Slope
HBOA	248	0.0551
HMBOA	256	0.0548
BOA	274	0.0362
MBOA	231	0.0637
M_2BOA	230	0.0519

was eluted from the silica gel with 5 ml of the 95% ethyl alcohol. The alcohol was allowed to evaporate. The residue was then taken up in 150 ul of 95% ethyl alcohol. Fifty ul of this solution was injected into a 5 ml volumetric flask and diluted to the mark with 95% ethyl alcohol.

This solution was used to obtain the UV absorbance values. Fifty ul of the original solution was injected into 20 ml of a liquid scintillation solution containing, 4 g of 2.5 - diphenyloxazole and 100 mg of 1,4-bis-(5-phenyloxazol-2-yl)-benzene diluted to one liter with toluene. The specific activity of each ^{14}C-labeled compound and the specific activity of the isolated materials was determined with a Nuclear Chicago Mark I ® liquid-scintillation spectrometer. A description of the basic techniques involved in an isotope silution analysis and the mathematical calculations necessary are given in Klun and Brindley (1966).

The spectrophotometric analysis developed for HMBOA followed the isolation procedure described above. The UV absorbance was read at 256 mμ and the concentration was calculated from the standard curve. The standard deviation for this technique was found to be ± 0.15 μ mol HMBOA/g dry weight tissue. The concentration data for a replicated chemical analysis are presented in Appendix Tables 79 and 80.

The supply of ^{14}C HBOA was used up before the third replicate of the greenhouse tissue chemical analyses were conducted. A spectrophotometric analysis of this tissue for HBOA was conducted. These data are presented in Appendix Tables 81 and 82.

Estimates of the reliability of the chemical analyses for the individual 1,4-benzoxazin-3 ones are represented by their standard errors derived from the chemical analyses of the greenhouse plant tissue. These data are presented in Appendix Table 83. These data give a realistic estimate of the error which might be encountered, since they are composed of variances in; tissue sampling procedures, inbred development rates, concentration changes due to time, concentration changes due to environmental conditions, and the analytical procedure.

A plate rating procedure was developed for separating tissue extracts by differences in their MBOA concentration. This procedure was not used in the chemical analyses reported in this research. Since this method has not been used previously, a description of the technique and a short discussion of the value of such a technique is presented in the following paragraphs.

An observation was made while viewing the chromatograms of the different inbreds under short-wave UV light, that there were noticeable, visual differences in the intensities of the spots corresponding to MBOA between the inbreds. The intensity of the spots, representing the inbreds, seemed to parallel their MBOA concentration (greater intensity = higher MBOA concentration). These inbred differences could be due to different

amounts of MBOA recovered in the analytical procedure. Based on these observations, the concentration of MBOA was calculated by using the isotope dilution technique and by using just the UV absorbance values. These data are presented in Table 4.

Table 4. MBOA concentration values for inbreds WF9, R101, W22, B52, Oh43 and CI31A from two analytical procedures

	Inbreds						Mean
	WF9	R101	W22	B52	Oh43	CI31A	
Spectrophotometric Analysis	1.20[a]	2.14	4.41	2.20	3.58	5.35	3.15
Isotope Dilution Analysis	1.15	2.09	4.32	2.09	3.20	5.67	3.09

[a]MBOA concentration expressed as mg MBOA/g dry whorl tissue.

Since there was a good agreement between these two analytical methods for MBOA concentrations, it suggested the possibility that a plate rating system could be developed which would group the inbreds according to their MBOA concentrations. This method could be of benefit in an initial DIMBOA screening program because of its quickness and relatively small investment in equipment.

A test was designed to determine if differences in MBOA concentrations between inbreds could be detected

visually, when chromatograms of inbred extracts were illuminated with short wave UV light. A standard plate was prepared by spotting four different amounts of MBOA on a single GF254 silica gel coated TLC plate. The plate was developed first in a chloroform-ethyl acetate-cyclohexane (4:4:2 v/v) solvent system and then all silica gel from one cm. below (toward the origin) the MBOA spot was removed from the plate. The plate was then developed in a cyclohexane-isobutanol (85:15 v/v) solvent system. Samples of dry whorl tissue from each of the inbreds WF9, R101, W22, B52, Oh43 and CI31A were extracted as described previously. A single spot for each inbred was placed on a single TLC plate and developed like the standard plate. The inbred extracts (spots) were randomized as to their position on the plate.

A plate rating system, based on the standard plate, was set up. Observers were asked to visually compare the intensity, under short wave UV light, of each inbred spot with the spots on the standard plate and to assign each inbred spot a value on the plate rating scale. The plate rating values and their corresponding amounts of MBOA on the standard plate are presented in Table 5.

In the initial test, six observers were asked to rate each inbred extract spot on the plate. The results from this test are presented in Table 6.

Table 5. Plate rating values and their corresponding amounts of MBOA

	Amount MBOA Spotted[a]				
	5.21	10.42	20.84	31.26	-
Plate rating value	1	2	3	4	5
Range of MBOA concentration for each plate rating value	0-5.21	5.22-10.42	10.43-20.84	20.85-31.26	>31.26

[a]µg MBOA spotted.

Table 6. Plate rating values and the actual amount of MBOA spotted for inbreds WF9, R101, W22, B52, Oh43 and CI31A

Inbred	µg MBOA spotted[a]	Plate Rating Values Observer						Mean
		1	2	3	4	5	6	
WF9	12.08	1	1	1	1	1	1	1.00
R101	17.64	1	2	2	2	1	1	1.50
W22	34.10	4	4	4	3	4	3	3.67
B52	18.80	1	2	2	2	2	1	1.67
Oh43	24.37	3	3	3	3	3	2	2.83
CI31A	41.06	4	4	4	4	4	3	3.83

[a]Actual µg MBOA spotted was determined by isotope dilution analysis.

These data show that the inbreds could be divided into two groups based on their plate rating values.

Extracts, two replicates, of each of the six inbreds were prepared. The MBOA concentration of each inbred extract was determined by spectrophotometric analysis and by isotope dilution analysis, and the intensity of each inbred spot was rated by each of three observers. These data and their corresponding analyses of variance are presented in Appendix Tables 84-89. The means of these data and their corresponding standard deviations and LSD's are presented in Table 7.

Table 7. Mean MBOA concentrations by spectrophotometric analysis and by isotope dilution analysis, and the mean plate rating values for six inbreds

Type of Analysis	Inbreds						Standard Deviation	LSD
	WF9	R101	W22	B52	Oh43	CI31A		
Spectro-photometric Analysis	0.77[a]	1.04	2.09	1.10	1.15	3.02	0.15	0.52[b]
Isotope Dilution Analysis	1.02	1.29	2.54	1.50	1.69	3.51	0.13	0.52[b]
Plate rating	1.00[c]	1.50	3.00	1.67	2.00	4.17	0.66	1.62[d]

[a]MBOA concentration expressed as mg MBOA/g dry tissue for spectrophotometric and isotope dilution analysis.

[b]LSD (.01).

[c]Plate rating values; values based on standard plate.

[d]LSD (.05).

These data show that a satisfactory plate rating system could be developed. This system would be faster and much less costly than either the isotope dilution analysis or the spectrophotometric analysis. The drawbacks to using such a system are: (1) plate rating would be less precise than either of the other techniques, and (2) much more careful spotting of the extracts on the plate is required. A plate rating system could be used effectively in a DIMBOA screening program to eliminate certain lines which have a low DIMBOA concentration and then a more precise chemical analysis could be used to separate the lines which have higher DIMBOA concentrations. This technique could possibly be used to rate commercial hybrids for their DIMBOA type resistance by comparing them with inbreds which have a known range of DIMBOA concentration.

Laboratory Tests

Two types of laboratory bioassay tests were conducted: (1) confinement tests - European corn borer larvae were confined to DIMBOA treated diets and allowed to develop, and (2) choice tests - (2 types) European corn borer larvae were given a choice of feeding on: (a) DIMBOA treated or untreated diet, and (b) DIMBOA treated or untreated WF9 leaf discs.

For the DIMBOA diet bioassay tests, DIMBOA was

incorporated into the European corn borer wheat germ diet (Lewis and Lynch, 1969) using the technique previously described by Klun et al., (1967). All laboratory bioassay tests were conducted in an incubator which had continuous light, constant temperature (26.5° C) and 80% relative humidity.

Confinement test

A confinement test was conducted to determine the effects of different DIMBOA dosage levels on the European corn borer as measured by: larval mortality, pupal weight and length of time to pupation. Single pair matings were made with the survivors to determine if larvae fed on a diet containing DIMBOA would have any detrimental effects on total egg mass production of the resulting adults.

The levels of DIMBOA tested were; 25.28, 12.64, 6.32, 3.16, and 1.58 μ mol DIMBOA/g dry wt diet. A control containing no DIMBOA was included in the test. Three gram, 15 mm dia diet plugs were placed in sterilized 17-mm dia x 65-mm length shell vials. One first instar European corn borer larva was placed in each vial. The vial was plugged with sterilized cotton and placed in the incubator. The vials were observed daily and as the larvae pupated, the pupae were removed from the vials and the length of time to pupation and the pupal weight were recorded. Each pupa was

placed into a two oz plastic jelly cup containing a wet strip of blotter paper. The sex of the emerged adults was recorded. Single pair matings were made using the technique previously described by Reed et al., (1972). An attempt was made to obtain 25 matings/dosage level. The total number of egg masses produced/mating was recorded. The egg masses were allowed to hatch and a visual assessment of 50% or greater hatch was recorded.

The confinement test was conducted as a randomized complete block design with five replications and 17 larvae/level/replication. The mating study was designed as a completely random design with unequal replication.

Choice tests

Choice tests were designed to determine if DIMBOA had any effect on the feeding behavior of European corn borer larvae. These tests were conducted in choice test arenas constructed from 100-mm dia x 15-mm height disposable, plastic, petri dishes. Melted paraffin (50 ml) was poured into the bottom of the petri dish. After solidification, individual feeding stations for the assay materials were made by cutting four evenly spaced 16-mm dia x 12-mm depth holes in the paraffin. To minimize larval escapes, 30 ml of melted paraffin were poured into the top of each petri dish. After the paraffin solidified, a tight fitting seal was obtained by forcing the

petri dish halves together.

For the choice tests conducted with artificial diet, the DIMBOA was mixed with the diet as described previously. Diet plugs, 15-mm dia x 6-mm height, were cut and placed in the feeding stations. Two DIMBOA treated plugs and two untreated plugs were placed in each arena. The dosage levels tested were; 25.28, 12.64, 6.32, 3.16, 1.58, 0.79 and 0.39 μ mol DIMBOA/g dry wt diet. For the choice tests conducted with WF9 leaf discs, a solution containing 1 mg DIMBOA/ml water was prepared. Fifteen mm dia leaf discs were cut from WF9 whorl leaves. The leaf discs were placed in the DIMBOA solution and agitated for five minutes. The leaf discs were removed from the solution and air dried. Control leaf discs were prepared by placing leaf discs in distilled water, agitating for five minutes and then air drying. Two DIMBOA treated leaf discs and two untreated leaf discs were placed in each arena.

Within an arena, the assay materials (treated and control) were randomly assigned to the four feeding stations. European corn borer larvae (10, one-day old larvae/arena or 5, five or ten day old larvae/arena) were then placed in the center of each arena, and the arena was sealed. To minimize the effects of light, the arenas were placed in 12-cm dia x 24-cm height stainless steel petri dish holders. The holders were capped and then placed in the incubator. At the conclusion of a test, the number of larvae at each feeding station and the

number of larvae on the arena surface were recorded. Several choice tests were conducted in this manner.

DIMBOA Spray Tests

Two DIMBOA spray tests were conducted. In both tests, a hand-held atomizer was used to spray a solution containing 1 mg DIMBOA/ml water in the whorl portion of susceptible plants. In each test distilled water was applied to separate plants as a control.

In the first test, 12 WF9 field grown plants (ca. 80 cm in extended leaf height) were transplanted into pots and brought into the greenhouse. Six plants were treated with 6 ml of DIMBOA solution per plant and six plants were treated with 6 ml of distilled water per plant. The number of larvae trapped at the end of 24 hours was recorded.

In the second test, eight plants (WF9 x M14; a susceptible single cross), were grown in the greenhouse. When these plants reached ca 80 cm in extended leaf height, four plants were treated with 2 ml of DIMBOA solution per plant and four plants were treated with 2 ml of distilled water. The number of larvae established and the number of larvae trapped were recorded at the end of 4 days.

RESULTS AND DISCUSSION

A summary of the results of the individual analyses of variance for the greenhouse tests and the field test are presented in Table 8. In all tests, larval establishment and migration were significantly different between inbred lines. Differences in larval mortality between inbreds

Table 8. Statistical significance of inbred differences from individual analyses of variance for each test and each data set

Test Number and Year[a]	Mean Number of Larvae Established	Mean Number of Larvae Trapped	Mean Number of Dead Larvae	Mean Weight (mg) of Surviving Larvae
1-1969	-**	-**	-**	-**
2-1969	-**	-*	-*	-**
3-1970	-**	-**	n.s.	-**
4-1970	-**	-**	-**	-**
5-1970	-*	-**	n.s.	n.s.
6-1971	-*	-**	-*	-

*Significant at the 95% probability level; this symbol used throughout the text.

**Significant at the 99% probability level; this symbol used throughout the text.

were significant for tests 1, 2, 4 and 6. Average weight per surviving larva was significant between inbreds in tests 1-4. These data indicate that larval establishment and migration are more stable criteria for measuring inbred dif-

ferences than either larval mortality on the plant or average weight per surviving larva. The data, analyses of variance and LSD's for each test and each data set are presented in Appendix Tables 26-54.

The suitability of a particular inbred line as a host for early instar European corn borer larvae is indicated by the number of larvae established and the average weight per larva of the survivors. These data means are presented in Tables 9 and 10. These data show that there are consistent

Table 9. Mean number of larvae established per plant[a]

Inbred	Test Number and Year					
	1-1969	2-1969	3-1970	4-1970	5-1970	6-1971
WF9	25.1a	22.7a	21.8a	21.4a	13.5ab	9.0a
R101	--	--	23.4a	12.4bc	14.7ab	8.7a
W22	--	--	11.4bc	8.8c	7.0bc	6.7a
B52	--	--	14.4b	8.4c	15.8a	5.3ab
Oh43	6.5b	12.8b	8.8c	13.8b	11.0ab	5.3ab
CI31A	2.6c	11.9b	2.0d	2.6d	1.8c	2.3b

[a]Initial population 30 larvae/plant; -- = inbred not included in test; means followed by same letter are not statistically significant at the 95% level of probability; letters indicating differences are valid only for test number under which they are listed.

Table 10. Mean weight (mg) per surviving larva[a]

Inbred	Test Number and Year				
	1-1969	2-1969	3-1970	4-1970	5-1970
WF9	0.77a	0.77a	0.80a	0.58a	0.69
R101	--	--	0.74ab	0.61a	0.80
W22	--	--	0.45cde	0.25b	0.47
B52	--	--	0.51bcd	0.48a	0.55
Oh43	0.36b	0.62ab	0.55abc	0.48a	0.63
CI31A	0.35b	0.47b	0.24e	0.31b	0.45

[a]-- = inbred not included in test; means followed by same letter are not statistically significant at the 95% level of probability; letters indicating differences are valid only for test number under which they are listed; inbred differences calculated only for tests with a significant F-ratio; weight of survivors not taken in test 6.

differences between inbreds in the number of larvae established and the average weight per larva between the highly susceptible, WF9, and the highly resistant, CI31A. The response of the inbreds whose resistance level lies between these two extremes is more variable, as would be expected.

Differences between inbreds in the number of larvae established actually reflect differences between inbreds in larval mortality. For these studies, the subtractive influence of mortality was divided into two categories, larval migration off the plant, and larval mortality on the plant. Larval migration, by early instar larvae, can be considered a mor-

tality factor since in most field situations the larvae would move to plants having the same level of resistance. The migrating larvae would also be more susceptible to environmental hazards. These data means are presented in Tables 11 and 12. These data show that there are significant

Table 11. Mean number of larvae trapped per plant[a]

Inbred	Test Number and Year					
	1-1969	2-1969	3-1970	4-1970	5-1970	6-1971
WF9	0.6a	1.4a	2.4ab	0.4ab	0.3a	3.0a
R101	--	--	1.4a	0.0a	0.7a	3.7a
W22	--	--	6.4b	2.2ab	4.2a	6.7b
B52	--	--	4.0ab	2.4ab	1.8a	3.3a
Oh43	11.9b	5.6b	10.8c	3.4b	3.6a	3.0a
CI31A	13.5b	5.5b	18.8d	11.0c	12.2b	12.3c

[a]Initial population 30 larvae/plant; -- = inbred not included in test; means followed by same letter are not statistically significant the 95% level of probability; letters indicating differences are valid only for test number under which they are listed.

differences in the number of larvae migrating off the highly resistant inbred, CI31A, when compared to the highly susceptible inbred, WF9. In general, there were more larvae migrating off the resistant and intermediate plants (Oh43, W22 and B52), than off the susceptible plants (WF9 and R101). Larval mortality on the plant was the least consistent data

Table 12. Mean number of dead larvae per plant[a]

Inbred	Test Number and Year					
	1-1969	2-1969	3-1970	4-1970	5-1970	6-1971
WF9	4.3a	5.9a	5.8	8.2a	16.2	18.0ab
R101	--	--	5.2	17.6c	14.6	17.7ab
W22	--	--	12.2	19.0c	18.8	16.7b
B52	--	--	11.6	19.2c	12.6	21.3a
Oh43	11.6b	11.6b	10.4	12.8b	15.4	21.7a
CI31A	13.9b	12.6b	9.2	16.4bc	16.0	15.3b

[a]Initial population 30 larvae/plant; -- = inbred not included in test; means followed by same letter are not statistically significant at the 95% level of probability; letters indicating differences are valid only for test number under which they are listed; inbreds differences calculated only for tests with a significant F-ratio.

taken. Generally, WF9 had the lowest larval mortality on the plant and the intermediates, W22 and B52, had the highest larval mortality on the plant. However, excluding WF9, there is little difference in larval mortality on the plant between the other inbred lines included in this study.

The relationship between larval establishment and larval migration and the relationship between larval establishment and larval mortality on the plant were examined by simple correlation. Correlation coefficients were calculated for all the individual plant data and for each inbred line. These

correlation coefficients were calculated from the individual plant data collected in the greenhouse (Tests 1-5) and are presented in Table 13. Each overall correlation coefficient is highly significant, which indicates that both larval migration off the plant and larval mortality on the plant play an important role in larval establishment. The overall correlation coefficients show the relationships for the group of

Table 13. Correlation coefficients between migration and establishment, and between mortality and establishment, calculated from individual greenhouse plant data collected in 1969 and 1970 (Tests 1-5)

	Overall[a]	Inbreds[b]					
		WF9	R101	W22	B52	Oh43	CI31A
Migration	-0.62**[c]	0.02	0.16	-0.12	-0.09	-0.46*	-0.60**
Mortality	-0.68**	-0.95**	-0.99**	-0.72**	-0.96**	-0.42*	-0.25

[a]All individual plant data from greenhouse tests in 1969 and 1970 (Tests 1-5).

[b]Inbred data for WF9, Oh43 and CI31A from greenhouse tests in 1969 and 1970 (Tests 1-5); inbred data for R101, B52 and W22 from greenhouse tests in 1970 (Tests 3-5).

*Significant at the 95% level of probability.

**Significant at the 99% level of probability.

inbred lines included in this study. The individual inbred line correlation coefficients show the relationship between each data set for a particular inbred line. The individual correlation coefficients indicate that larval mortality on the plant is highly correlated with larval establishment on the

susceptible inbreds, WF9 and R101, and the intermediate inbreds, W22 and B52. For the resistant inbred line, Oh43, larval migration off the plant and larval mortality on the plant are both significantly correlated with larval establishment. However, in the highly resistant inbred line, CI31A, larval migration alone is highly correlated with larval establishment.

These relationships bring up an important question, what component (mechanism) of resistance is acting in first generation European corn borer host plant resistance? First generation resistance has been termed antibiosis (Brindley and Dicke, 1963; Reed et al., 1972) and has been characterized by a high mortality of early instar larvae (Guthrie et al., 1960) and by larger leaf feeding scars on susceptible versus resistant plants (more feeding/larva on susceptible tissue) (Chin, 1951; Dicke and Penney, 1954). However, resistance to larval feeding is probably resistance of the nonpreference type, therefore first generation resistance is composed of both antibiosis and nonpreference (Beck, 1965). From the studies reported here, if an average percent mortality on the plant for each inbred line across tests (Tests 1-6) is computed, the values are WF9, 32%; R101, 46%; W22, 56%; B52, 54%; Oh43, 46%; and CI31A, 46%. These data show that there is little difference between inbreds in percent mortality on the plant, except for WF9. However, if an average percent migration off the plant for each inbred line is computed across

tests (Tests 1-6) the values are WF9, 5%; R101, 5%; W22, 16%; B52, 10%; Oh43, 21%; and CI31A, 41%. These data show that there are distinct differences between inbreds in percent larval migration off the plant. Since larval migration off the plant is a behavioral reaction, first generation European corn borer host plant resistance could be explained as resistance of the nonpreference type. Certainly, the high degree of resistance found in the inbred, CI31A, can be explained as an increase in the nonpreference component of resistance when compared to the other inbred lines in this study.

The average weight per surviving larva suggests that some antibiotic effects are present since the survivors on CI31A weighed less than the survivors on WF9. However, this response could be a behaviorally mediated response due to a higher concentration of a feeding deterrent present in the resistant plant. Although the presence of a feeding deterrent is plausible, several other factors such as nutrient deficiencies or nutrient imbalances between inbred lines could account for these differences.

Previous studies have evaluated first generation resistance as differences in larval establishment (survivors). An evaluation of this type does not distinguish between mortality factors and gives little evidence for any component of resistance other than antibiosis. In a

practical host plant resistance breeding program this approach is realistic since all that is required is that a character for resistance be inherited. However, by separating out lines which have a high level of nonpreference and lines which have a high level of antibiosis, it may be possible to combine these two components to produce a more resistant plant.

Previously, whorl concentrations of DIMBOA were shown to be significantly related to first generation European corn borer field resistance ratings (Klun and Brindley, 1966; Klun and Robinson, 1969; Klun et al., 1970). DIMBOA was bioassayed in an artificial diet and found to be biologically active against the European corn borer. It was suggested that DIMBOA was acting as a feeding deterrent and/or repellent (Klun et al., 1967). One of the primary objectives of the research reported here was to investigate the relationships between whorl concentrations of DIMBOA, and larval establishment, larval migration off the plant, and larval mortality on the plant. Due to the development of a two-dimensional TLC procedure, (designed specifically for MBOA determinations), concentration differences between inbred lines for four homologs of DIMBOA (HOBA, HMBOA, DIBOA, DIM_2BOA) were investigated and the relationship between the concentration of each homolog and the biological data was also

evaluated.

The means of the biological and the 1,4-benzoxazin-3-one concentration data for tests conducted in the greenhouse (Tests 3-5) are presented in Table 14. Table 15 presents the correlation coefficients between the biological and the concentration data for these tests. The means of the biological and the 1,4-benzoxazin-3-one concentration data for the field test are presented in Table 16. Correlation coefficients between the biological and the concentration data for the field test are presented in Table 17. Since the 1,4-benzoxazin-3-one concentrations for the greenhouse plants were determined for an aggregate sample of whorl tissue for each inbred within a test, combined analyses of variance of the average biological data were calculated for each data set. The average performance of each inbred within a test served as a replicate. The concentration data for the greenhouse plants, analyses of variance, and the combined (Tests 3-5) analyses of variance of the average biological data are presented in Appendix Tables 55-68. The 1,4-benzoxazin-3-one concentrations for the field plants and the analyses of variance of this data are presented in Appendix Tables 69-78. The field biological data (Test 6) and the analyses of variance of these data are presented in Appendix Tables 51-54.

Table 14. Average resistance ratings, mean number of larvae established, mean number of larvae on the targets, mean number of dead larvae, and mean 1,4-benzoxazin-3-one concentrations

	Inbreds						
	WF9	R101	W22	B52	Oh43	CI31A	LSD (.05)[a]
Average Resistance Rating[b]	8	8	6	6	4	2	-
Mean Weight (mg) Surviving Larvae[c]	0.69	0.72	0.39	0.51	0.55	0.33	0.09
Mean Number of Larvae Established	18.90	16.82	9.07	12.87	11.47	2.13	6.73
Mean Number of Larvae on Traps	1.02	0.69	4.27	2.73	5.37	14.00	3.45
Mean Number of Dead Larvae	10.08	12.49	16.67	14.40	13.30	13.87	-
Mean μ mol HBOA	1.74	2.65	3.13	2.18	2.32	2.75	-
Mean μ mol HMBOA	0.89	1.16	1.83	1.25	1.15	3.08	1.00
Mean μ mol DIBOA	3.19	4.00	3.30	3.23	3.94	4.88	-
Mean μ mol DIMBOA	6.43	10.06	19.25	9.97	13.56	25.77	4.79
Mean μ mol DIM_2BOA	5.24	6.83	4.62	4.32	3.91	4.22	-

[a]LSD = least significant difference; calculated only for tests with a significant F-ratio.

[b]Field resistance ratings from Guthrie and Dicke (1972).

[c]Means from greenhouse tests three, four and five (1970).

Table 15. Correlation coefficients between 1,4-benzoxazin-3-one concentrations, and the biological data (1970)[a]

	1,4-benzoxazin-3-one				
	HBOA	HMBOA	DIBOA	DIMBOA	DIM_2BOA
Average Resistance Rating	-0.34[b]	-0.78	-0.72	-0.82	0.73
Number of Larvae Established	-0.62	-0.93	-0.67	-0.96	0.60
Number of Larvae on Traps	0.40	0.93	0.80	0.90	-0.56
Number of Dead Larvae	0.80	0.45	0.02	0.61	-0.39
Average Weight (mg) per Surviving Larva	-0.60	-0.84	-0.37	-0.89	0.71

[a]Means of the greenhouse data (Tests 3-5, 1970).

[b]Data presented are correlation coefficients; values equal to or greater than 0.81 and 0.92 are significant at the 95% and 99% levels of probability, respectively.

For the greenhouse data, the combined analyses of variance of the biological data show significant differences between inbreds for weight of surviving larvae, number of larvae established, and number of larvae on the traps. Larval mortality on the plant was not significantly different between the inbreds for this analysis. Significant differences were found between inbreds in the field test for larval establishment, larval migration off the plant, and larval mortality on the plant.

Table 16. Average resistance ratings, mean number of larvae established, mean number of larvae on traps, mean number of dead larvae and mean 1,4-benzoxazin-3-one concentrations (1971)

	Inbreds						
	WF9	R101	W22	B52	Oh43	CI31A	LSD (.05)[c]
Average Resistance Rating[a]	8	8	6	6	4	2	-
Mean Number of Larvae Established[b]	9.00	8.67	6.67	5.33	5.33	2.33	4.00
Mean Number of Larvae on Traps	3.00	3.67	6.67	3.33	3.00	12.33	2.30
Mean Number of Dead Larvae	18.00	17.67	16.67	21.33	21.67	15.33	4.30
Mean μ mol HBOA	4.16	4.30	5.34	3.45	5.03	3.94	-
Mean μ mol HMBOA	1.36	2.37	1.92	2.02	1.96	3.45	-
Mean μ mol DIBOA	3.29	4.38	4.70	4.83	4.62	4.11	-
Mean μ mol DIMBOA	6.40	12.63	13.44	10.12	12.73	23.01	4.01
Mean μ mol DIM_2BOA	5.04	6.62	5.98	4.32	6.08	3.90	-

[a] Field resistance ratings from Guthrie and Dicke (1972).

[b] Means from field (Test 6, 1971).

[c] LSD = least significant difference; calculated for biological data and significant (F-ratio) concentration data.

Table 17. Correlation coefficients between 1,4-benzoxazin-3 one concentrations, and the biological data (1971)[a]

	Average Resistance Rating	Mean Number of Larvae Established	Mean Number of Larvae on Traps	Mean Number of Dead Larvae
HBOA	-0.02[b]	0.18	-0.06	-0.09
HMBOA	-0.72	-0.75	0.85	-0.50
DIBOA	-0.26	-0.35	-0.04	0.39
DIMBOA	-0.83	-0.81	0.91	-0.54
DIM_2BOA	0.49	-0.40	-0.52	0.14

[a]Means of the field data (Test 6, 1971).

[b]Data presented are correlation coefficients; values equal to or greater than 0.81 are significant at the 95% level of probability.

Correlation coefficients between the greenhouse and field data were 0.92** for larval establishment, 0.90* for larval migration, and -0.09 for larval mortality on the plant. These data indicate strong relationships between the greenhouse tests and field test for larval establishment and larval migration. Larval mortality on the plant was not significantly correlated between the greenhouse tests and the field test. This lack of correlation may be explained by differences in environmental conditions between

the tests.

Correlation coefficients between the average field leaf feeding resistance ratings and the means of the biological data from the greenhouse tests and from the field test were 0.91* (greenhouse) and 0.96** (field) for larval establishment, -0.94** (greenhouse) and -0.72 (field) for larval migration, and -0.39 (greenhouse) and 0.12 (field) for larval mortality on the plant. These results show a significant correlation between larval establishment, for both the greenhouse and field tests, and the average field resistance ratings. Larval migration in the greenhouse tests was significantly correlated to the resistance ratings but this relationship was not significant for the field data. The relationship between larval mortality on the plant and resistance ratings was not significant for either the greenhouse data or the field data. These data indicate that the results found in the greenhouse tests and the field test are consistent with the average leaf feeding resistant ratings reported by Guthrie and Dicke (1972). Larval migration off the plant is better correlated with leaf feeding ratings than is larval mortality on the plant.

Significant differences between inbreds for DIMBOA concentration were found for both the greenhouse and field tests. The average DIMBOA concentrations (Tests 3-6) were higher

than the DIMBOA concentrations reported by Klun et al. (1970). However, the correlation coefficient between the DIMBOA concentrations found by Klun et al. (1970) and the DIMBOA concentrations found in this study was 0.98**. This highly significant correlation coefficient indicates that whatever factors influence the changes in plant concentration of DIMBOA from one chemical analysis to the next effect each inbred line in the same manner. HMBOA concentration was significantly different between inbreds in the greenhouse test but was not significantly different in the field test. No significant differences between inbreds for HBOA, DIBOA and DIM_2BOA concentrations were found in either test.

Tables 18 and 19 present the average 1,4-benzoxazin-3-one concentrations and the u mol percents of each compound for the six inbred lines. The data presented are an average of five chemical analyses. These data show that an inbred line can differ in both the concentration of a particular chemical present and the percentage of the total 1,4-benzoxazin-3-one composition each chemical represents.

The correlation coefficients (Tables 15 and 17) indicate that DIMBOA concentration is significantly correlated with the number of larvae established, the number of larvae on the traps and the average field resistance ratings, in both the greenhouse tests and the field test. Average weight of the

Table 18. Average concentration of five 1,4-benzoxazin-3-ones for six inbred lines of corn[a]

Inbred	1,4-Benzoxazin-3-one					Total
	HBOA	HMBOA	DIBOA	DIMBOA	DIM_2BOA	
WF9	2.71	1.08	3.23	6.42	5.16	18.60
R101	3.31	1.64	4.15	11.09	6.75	26.94
W22	4.01	1.87	3.86	16.93	5.16	31.83
B52	2.69	1.56	3.87	10.03	4.32	22.47
Oh43	3.40	1.47	4.21	13.23	4.78	27.09
CI31A	3.23	3.23	4.57	24.67	4.09	39.79

[a]Concentration expressed as μ mol/g dry weight tissue; average of concentrations derived from five chemical analyses (3 analyses in 1970 and 2 analyses in 1971).

Table 19. Average μ mol percent of five 1,4-benzoxazin-3-ones for six inbred lines of corn[a]

Inbred	1,4-Benzoxazin-3-one				
	HBOA	HMBOA	DIBOA	DIMBOA	DIM_2BOA
WF9	14.57	5.81	17.37	34.51	27.74
R101	12.29	6.09	15.41	41.16	25.05
W22	12.60	5.88	12.13	53.18	16.21
B52	11.97	6.94	17.22	44.64	19.23
Oh43	12.55	5.43	15.54	48.84	17.64
CI31A	8.12	8.12	11.49	62.00	10.27

[a]Values presented are μ mol percents; derived from data in Table 18.

surviving larvae was also significantly correlated with DIMBOA concentrations. Larval mortality on the plant was not significantly correlated to DIMBOA concentrations in either test.

HMBOA concentrations were significantly correlated with the number of larvae established, the number of larvae trapped and the average weight of the surviving larvae in the greenhouse test. In the field test, HMBOA concentration was significantly correlated to the number of larvae trapped. However, these relationships are somewhat suspect since a 0.95** correlation coefficient existed between HMBOA concentrations and DIMBOA concentrations. This high correlation could be expected from the biosynthetic scheme proposed by Tipton et al. (1973). Also, Klun and Robinson (unpublished data, European corn borer Lab, Ankeny, Iowa) bioassayed HMBOA in a confinement test and found HMBOA had no European corn borer biological activity. However, HMBOA has not been bioassayed in a choice test and the possibilities do exist that it may be active in a choice test or that a combination of HMBOA and DIMBOA may be more active than either compound alone.

Since these studies have indicated that plant concentrations of DIMBOA are related to a nonpreference component of resistance, bioassay tests were conducted by incorporating DIMBOA into an artificial diet. Results of the DIMBOA con-

finement test are presented in Table 20. The data and the analyses of variance for these data are presented in Appendix Tables 90-94.

Table 20. Mean days to pupation and mean pupal weights for larvae confined to DIMBOA treated diet

DIMBOA[a] Level	Sex	Mean Days to Pupation	Mean Pupal Weight
25.28	Male	20.17	66.45
	Female	22.37	89.14
12.64	Male	18.21	70.26
	Female	19.69	96.28
6.32	Male	17.62	72.39
	Female	18.64	97.10
3.16	Male	17.07	76.65
	Female	18.53	103.42
1.58	Male	16.99	75.86
	Female	18.05	103.37
Control	Male	17.26	76.46
	Female	17.73	104.18
LSD (.01)	Male	0.91	5.93
	Female	0.71	6.22

[a]Level = μ mol DIMBOA/g dry wt diet.

No significant differences in mortality occurred in the confinement test. The overall mortality was 6.27 percent. These data are not in agreement with Klun et al. (1967) who reported a 25% mortality for a 0.32 mg DIMBOA/g diet (ca. 10.83 μ mol DIMBOA/g dry wt diet) concentration. The factors

responsible for the difference in mortality between these bioassays cannot be explained.

The analyses of variance (Tables 91-94) show that DIMBOA exerted a significant effect on length of time to pupation and pupal weights for both sexes. DIMBOA increased the length of time to pupation and decreased the pupal weights for both sexes. For the males, the 12.64 μ mol DIMBOA concentration was significantly different from the control for both the time to pupation and the pupal weight data. For the females, the 3.16 μ mol DIMBOA concentration and the 6.32 μ mol DIMBOA concentration significantly increased time to pupation and decreased pupal weights, respectively. These data indicate that the females were more sensitive to the DIMBOA concentrations than were the males.

Table 21 presents the mating data for the insects reared in the confinement test. These data indicate that rearing larvae on a diet containing DIMBOA had no significant effect on either mating or egg production of the resulting adults.

Reed et al. (1972) found that European corn borer larvae reared on resistant leaf tissue had greater mortality, developed slower, weighed less, and the resulting adults mated less successfully and produced fewer egg masses than insects reared on susceptible leaf tissue. The differences in the results between the DIMBOA confinement test and the leaf tissue feeding test (Reed et al., 1972) indicate that

Table 21. Matings from confinement test

Level[a]	Number of Matings	Number of Unmated Females	Percent Mating	Average Number Egg Masses Per Mating	Average Number Egg Masses 50% or Greater Hatch
25.28	19	7	63.2	22.2	14.9
12.64	21	3	85.7	21.4	16.0
6.32	25	9	64.0	15.1	12.9
3.16	23	7	69.6	20.4	16.3
1.58	25	7	72.0	19.6	16.2
Control	22	12	45.4	19.1	13.5
Average	22.5	7.5	66.7	19.6	15.0

[a]Level expressed as μ mol DIMBOA/g dry wt diet.

other factors can modify the expression of resistance. These factors may be independent of DIMBOA effects or they could increase or attenuate the effects of DIMBOA.

A series of choice tests were conducted to determine if European corn borer larvae could distinguish between DIMBOA treated diet and control diet. The results from this series of tests are presented in Table 22. All choice-test data and analyses of variance are presented in Appendix Tables 95-105.

Table 22. Mean counts for one, five and ten day old larvae, observed at four time periods, when given a choice between DIMBOA treated and control diets

Time of Observation (Hours)		Larval Age (Days) 1 Mean	1 t-value (48, df)	5 Mean	5 t-value (58, df)	10 Mean	10 t-value (58, df)
24	Control	5.56	9.73**	1.66	2.77**	1.37	3.61**
	Treatment[a]	1.92		1.03		0.63	
48	Control	5.52	9.94**	1.70	3.03**	1.57	3.21**
	Treatment	1.80		1.00		0.83	
92	Control	4.84	7.00**	2.13	4.66**	1.47	4.34**
	Treatment	2.04		0.83		0.60	
96	Control	3.84	6.02**	1.97	3.31**	1.20	3.10**
	Treatment	1.84		1.30		0.57	

[a] 12.64 μ mol DIMBOA/g dry wt diet.

** Significant at the 99% level of probability.

These data show that one, five and ten day old larvae can distinguish between DIMBOA treated and control diets. Based on these results, subsequent choice tests were conducted with one day old larvae and evaluated at the end of 24 hours.

Choice tests were conducted to determine at what level of DIMBOA first instar larvae could distinguish between treated and control diets. The data means from this series of tests are presented in Table 23.

Table 23. Mean number of larvae on control and treated diet, F-values and percent effect for individual choice tests

	Level[a]						
	0.39	0.79	1.58	3.16	6.32	12.64	25.28
Mean Control	18.67	22.33	22.00	28.00	24.67	26.00	29.33
Mean Treated	21.33	17.67	18.67	16.33	14.67	15.00	14.33
F-Value	0.31	1.70	1.35	5.20	6.04	9.59	15.00
Significance[b]	n.s.	n.s.	n.s.	-*	-*	-**	-**
Percent Effect	0.00	20.87	15.12	41.68	40.54	42.31	51.14

[a]Level = μ mol DIMBOA/g dry wt diet.

[b]n.s. = not significant.

*Significant at the 95% probability level.

**Significant at the 99% probability level; split-plot analysis; whole plots = runs; split-plots = level vs control; larval counts transformed to $\sqrt{x + 0.5}$.

These data indicate that one day old European corn borer larvae can distinguish between a diet containing 3.16 μ mol DIMBOA/g dry weight diet and a control diet. These results are somewhat consistent with the results obtained in the confinement test. In both tests, the lowest level of DIMBOA which had a significant effect was 3.16 μ mol DIMBOA/g dry weight diet. It is interesting to note that this level of DIMBOA is approximately one-half the level of DIMBOA found in WF9 whorl tissue. This could serve as further evidence that other factors such as environment,

nutrition or possibly other secondary chemicals could modify the effects of DIMBOA. Further bioassay tests (confinement and choice tests) with the other 1,4-benzoxazin-3-ones alone and in combinations with each other and with DIMBOA, at concentrations approaching those found in whorl tissue, may clarify the chemical nature of first generation resistance.

The results of the DIMBOA treated WF9 leaf discs tests are presented in Appendix Table 106. The significant F-value (5.26*) indicates that it might be possible to apply DIMBOA to susceptible plants to control the European corn borer. Two small spray tests were conducted to test this hypothesis. The results of these tests are presented in Tables 24 and 25.

The results from both spray tests show that application of DIMBOA to susceptible plants increases larval migration off the plant. However, in the second test in which the plants were dissected, no significant difference between the treatment and control for number of larvae established could be detected. The overall percent control, 18%, may have been too small to be detected with the number of plants included in the test. Overall, these tests indicate that some degree of protection could be given susceptible plants by the application of DIMBOA. However, more research needs to be done in the areas of formulation and application techniques before a workable system can be developed.

Table 24. DIMBOA spray test[a]

Plant Number	Number of Larvae Trapped	
	Control[b]	Treatment
1	1	1
2	0	1
3	1	3
4	0	6
5	1	9
6	0	3
Sum	3	23
Mean	0.50	3.83
F-Value	9.71*	

[a]All plants were WF9; 30 first instar larvae placed in the whorl of each plant; data recorded at the end of 24 hours.

[b]Control = 6 cc of distilled water/plant; treatment = 6 cc of a solution containing 1 mg DIMBOA/cc water/plant.

*Significant at the 95% level of probability; larval counts transformed to $\sqrt{x+0.5}$ and analyzed as a completely random design.

The results from the choice tests indicate that European corn borer larvae, when given a choice, prefer not to feed on DIMBOA treated diet or leaf discs. The increased time to pupation and the decreased pupal weights observed in the confinement test may be caused by this nonpreference response and not a toxic response per se. If the data from these bioassay tests can be extrapolated to the greenhouse and

Table 25. DIMBOA spray test[a]

Plant Number	Number of Larvae Trapped		Number of Larvae Established	
	Control[b]	Treatment	Control	Treatment
1	10	22	14	8
2	9	22	14	8
3	9	16	10	10
4	7	11	12	13
Sum	35	71	50	39
Mean	8.75	17.75	12.50	9.75
F-value	10.86*		4.63[c]	

[a]All plants were WF9XM14; 30 first instar larvae placed in the whorl of each plant; data recorded at the end of four days.

[b]Control = 2 cc of distilled water/plant; treatment = 2 cc of a solution containing 1 mg DIMBOA/cc water/plant.

[c]n.s. = not significant at the 95% level; analyzed as a completely random design.

*Significant at the 95% level of probability.

field tests, there is a good possibility that the mode of DIMBOA action against the European corn borer is a behavioral reaction of the nonpreference type. The results obtained in the greenhouse tests, field test and DIMBOA spray tests support this interpretation. This research indicates that for the inbred lines included in these studies, a non-preference component of resistance is present in first generation European corn borer resistance and that plant

concentration of DIMBOA is a primary agent in the expression of this nonpreference component of resistance.

CONCLUSIONS

The objectives of this study were to further elucidate the nature of first generation European corn borer host-plant resistance, in terms of larval establishment, larval mortality on the plant, and larval movement off the plant. The relationship between these biological phenomena and the plant concentration of DIMBOA were evaluated. Inbred concentration differences for four homologs of DIMBOA (HBOA, HMBOA, DIBOA, and DIM_2BOA) were also determined and the relationships between these homologs and the biological data were determined. Bioassay and spray tests with DIMBOA were conducted in an attempt to determine the nature of DIMBOA effects on early instar European corn borer larvae. From the results obtained in this research, the following conclusions were drawn:

1. A nonpreference component of resistance, as indicated by larval migration off the host plant, plays an important role in first-generation European corn borer resistance.

2. Larval mortality on the plant was not a significant factor in explaining first generation resistance for the inbred lines included in this research.

3. The high level of resistance found in the inbred line, CI31A, can be explained by its increased level of non-

preference when compared to the other inbred lines included in this study.

4. Significant differences between inbreds for DIMBOA concentrations were found in both the 1970 and 1971 chemical analyses. HMBOA concentration was significantly different between inbreds for the 1970 analysis but was not significantly different in the 1971 analysis. No significant differences between inbreds for HBOA, DIBOA and DIM_2BOA were found in either year.

5. Significant correlation coefficients were found between DIMBOA concentration and larval migration off the plant, larval establishment, average weight of the surviving larvae and the average field leaf feeding resistance ratings. DIMBOA concentrations were not significantly correlated to larval mortality on the plant. HMBOA concentrations were significantly correlated to larval establishment, larval migration off the plant and average weight of surviving larvae, in the 1970 test. In the 1971 test, HMBOA concentration was significantly correlated to larval migration off the plant. However, HMBOA concentrations were significantly correlated to DIMBOA concentrations, since HMBOA and DIMBOA have been shown to be related in their biosynthetic pathway and a previous bioassay of HMBOA showed no biological activity, these significant relationships are somewhat suspect. No significant correlations between the concentration

of HBOA, DIBOA and DIM_2BOA and the biological data were found.

6. A confinement bioassay test showed an increase in the length of time to pupation and a decrease in pupal weights for both sexes as the level of DIMBOA increased. For the males, the 12.64 μ mol DIMBOA/g dry weight diet concentration significantly increased time to pupation and decreased pupal weights when compared to the control. For the females, the 3.16 μ mol DIMBOA g dry weight diet concentration significantly increased time to pupation and the 6.32 μ mol DIMBOA g dry weight diet concentration significantly reduced pupal weights when compared to the control. No significant mortality effects were observed in this test. A mating study indicated that rearing larvae on DIMBOA treated diet had no effect on egg-mass production or egg hatch of the resulting adults.

7. Choice tests show that European corn borer larvae prefer not to feed on DIMBOA treated diet or DIMBOA treated WF9 leaf discs. For the diet tests, a significant difference between treated and control diet was observed with the 3.16 μ mol DIMBOA/g dry weight diet concentration.

8. Application of DIMBOA to susceptible plants significantly increased larval migration off the plant in two tests. For the second test, larval establishment data were taken and no significant differences between treated

and control plants were observed. However, DIMBOA treated plants did have lower establishment than untreated plants (18% control) and with the proper formulation, application techniques and dosage level, DIMBOA could possibly be used as a European corn borer control agent.

9. A plate rating technique was developed for separating inbred lines by differences in their MBOA concentration. This technique may prove to be useful in large scale screening tests.

The expression of host plant resistance in a cultivar is a complex of interwoven factors and plant concentration of DIMBOA is but one of these factors. However, the data collected in this research indicate that for the inbred lines included in this study, a nonpreference component of resistance is present in first generation European corn borer host plant resistance and that plant concentration of DIMBOA is a primary agent in the expression of this nonpreference component of resistance.

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APPENDIX

Table 26. Greenhouse (Test 1) 1969. Extended leaf height, tassel height, tassel bud ratio, number of larvae on traps (daily), number of larvae established, larval mortality and average weight of survivors on inbreds WF9, Oh43 and CI31A

Inbred	Plant No.	Extended Leaf Height (cm)	Tassel Height (cm)	Tassel Bud Ratio	Number of Larvae on Trap Day 1	Day 2	Day 3	Day 4	Sum
CI31A	1	85.1	10.2	12.0	6	1	0	0	7
	2	94.0	6.4	6.8	3	3	2	0	8
	3	86.4	11.4	13.2	5	0	1	0	6
	4	82.6	7.1	8.6	11	2	0	0	13
	5	92.2	9.7	10.5	20	4	1	0	25
	6	88.9	12.2	13.7	23	0	0	0	23
	7	86.4	7.1	8.2	6	1	2	1	10
	8	83.8	7.1	8.5	8	5	3	0	16
	Sum	699.4	71.2	81.5	82	16	9	1	108
	Mean	87.4	8.9	10.2	10.3	2.0	1.1	0.1	13.5
Oh43	1	88.9	10.2	11.5	10	0	0	0	10
	2	90.2	10.2	11.3	13	4	1	0	18
	3	92.7	10.2	11.0	13	0	1	0	14
	4	94.0	10.2	10.9	4	0	0	0	4
	5	99.1	10.9	11.0	17	2	1	0	20
	6	94.0	12.2	13.0	10	2	2	0	14
	7	94.0	10.2	10.9	8	0	2	0	10
	8	87.6	7.6	8.7	2	1	2	0	5
	Sum	740.5	81.7	88.3	77	9	9	0	95
	Mean	92.6	10.2	11.0	9.6	1.1	1.1	0	11.9
WF9	1	91.4	7.6	8.3	1	0	0	0	1
	2	91.4	5.1	5.6	0	0	0	0	0
	3	87.6	10.2	11.6	0	1	0	0	1
	4	88.9	6.4	7.2	1	0	0	0	1
	5	83.8	5.1	6.1	0	0	0	0	0
	6	82.6	7.1	8.6	0	0	0	0	0
	7	87.6	6.4	7.3	1	0	0	0	1
	8	83.8	6.4	7.6	0	1	0	0	1
	Sum	697.1	54.3	62.3	3	2	0	0	5
	Mean	87.1	6.8	7.8	0.4	0.3	0	0	0.6

Number Larvae Established	Average wt (mg) Survivors	Mortality (number larvae)
6	0.44	17
0	-	22
3	0.32	21
3	0.33	14
2	0.50	3
0	-	7
2	0.23	18
5	0.27	9
21	2.09	111
2.6	0.35	13.9
10	0.50	10
6	0.28	6
7	0.38	9
4	0.29	22
2	0.25	8
4	0.30	12
11	0.53	9
8	0.34	17
52	2.87	93
6.5	0.36	11.6
23	0.65	6
20	0.73	10
26	0.84	3
23	0.55	6
26	0.92	4
26	0.96	4
28	0.76	1
29	0.76	0
201	6.17	34
25.1	0.77	4.3

Table 27. Analysis of variance of number of larvae established on inbreds WF9, Oh43, and CI31A (greenhouse (Test 1) 1969)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	2	2315.1	1157.6	150.3**
Error	21	160.7	7.7[a]	
Total	23	2475.8		

[a]L.S.D. (.05) = 2.9.

**Significant at the 99% probability level.

Table 28. Analysis of variance of number of larvae on traps from inbreds WF9, Oh43 and CI31A (greenhouse (Test 1) 1969)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	2	786.6	393.3	13.8**
Error	21	600.6	28.6[a]	
Total	23	1387.2		

[a]L.S.D. (.05) = 5.6.

**Significant at the 99% probability level.

Table 29. Analysis of variance of larval mortality on inbreds WF9, Oh43, and CI31A (greenhouse (Test 1) 1969)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	2	405.6	202.8	7.1**
Error	21	600.2	28.6[a]	
Total	23	1005.8		

[a]L.S.D. (.05) = 5.6.

**Significant at the 99% probability level.

Table 30. Analysis of variance of average weight of surviving larvae on inbreds WF9, Oh43 and CI31A (Greenhouse (Test 1) 1969)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	2	0.89	0.45	45.00**
Error	19	0.25	0.01[a]	
Total	21	1.14		

[a]L.S.D. (.05) = WF9 or Oh43 vs CI31A 0.10; WF9 vs Oh43 0.10.

**Significant at the 99% probability level.

Table 31. Greenhouse (Test 2) 1969. Extended leaf height, tassel height, tassel bud ratio, number of larvae on traps (daily), number of larvae established, larvae mortality and average weight of survivors on inbreds WF9, Oh43 and CI31A

Inbred	Plant No.	Extended Leaf Height (cm)	Tassel Height (cm)	Tassel Bud Ratio	Number of Larvae on Traps				
					Day 1	Day 2	Day 3	Day 4	Sum
CI31A	1	94.0	10.2	10.9	3	0	0	0	3
	2	96.5	11.4	11.8	5	0	1	0	6
	3	104.1	16.5	15.9	1	3	2	0	6
	4	99.1	17.8	18.0	6	1	2	0	9
	5	99.1	12.4	12.5	5	0	0	0	5
	6	99.1	14.0	14.1	2	0	1	0	3
	7	99.1	16.5	16.6	3	2	2	0	7
	8	99.1	15.2	15.3	3	0	2	0	5
	Sum	790.1	114.0	115.1	28	6	10	0	44
	Mean	98.8	14.3	14.4	3.5	0.8	1.3	0	5.5
Oh43	1	109.2	25.4	23.3	4	0	0	0	4
	2	104.1	27.9	26.8	1	2	4	0	7
	3	106.7	27.9	26.2	4	1	1	0	6
	4	111.8	34.3	30.7	2	0	0	0	2
	5	106.7	25.4	23.8	4	5	3	0	12
	6	111.8	33.0	29.5	4	0	0	0	4
	7	111.8	25.4	22.7	6	1	1	0	8
	8	104.1	22.9	22.0	0	0	2	0	2
	Sum	866.2	87.5	205.0	25	9	11	0	45
	Mean	108.3	10.9	25.6	3.1	1.1	1.4	0	5.6
WF9	1	109.2	20.3	18.6	2	0	0	0	2
	2	101.6	16.5	16.2	0	0	0	0	0
	3	104.1	11.4	11.0	0	0	0	0	0
	4	101.6	10.1	9.9	6	1	0	0	7
	5	101.6	14.0	13.8	0	0	0	0	0
	6	104.1	17.8	17.1	1	0	0	0	1
	7	104.1	17.8	14.6	0	0	0	0	0
	Sum	726.3	41.5	101.27	9	1	0	0	10
	Mean	103.8	5.9	14.5	1.3	0.1	0	0	1.4

Number Larvae Established	Average wt (mg) Survivors	Mortality (number larvae)
15	0.55	12
9	0.39	15
13	0.60	11
12	0.37	9
3	0.43	22
20	0.50	7
10	0.43	13
13	0.52	12
95	3.79	101
11.9	0.47	12.6
13	0.70	13
9	0.54	14
10	0.54	14
10	0.82	18
14	0.70	4
23	0.59	3
12	0.58	10
11	0.48	17
102	4.95	93
12.8	0.62	11.6
25	0.92	3
24	0.79	6
25	0.84	5
19	0.75	4
25	0.75	5
14	0.52	15
27	0.80	3
159	5.37	41
22.7	0.77	5.9

Table 32. Analysis of variance of number of larvae established on inbreds WF9, Oh43 and CI31A (greenhouse (Test 2) 1969)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	2	529.9	265.0	12.2**
Error	20	433.8	21.7[a]	
Total	22	963.7		

[a]L.S.D. (.05) = CI31A or Oh43 vs WF9 5.0; CI31A vs Oh43 4.8.

**Significant at the 99% probability level.

Table 33. Analysis of variance of number of larvae on traps from inbreds WF9, Oh43 and CI31A (greenhouse (Test 2) 1969)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	2	84.3	42.2	5.8*
Error	20	146.6	7.3[a]	
Total	22	230.9		

[a]L.S.D. (.05) = CI31A or Oh43 vs WF9 2.9; CI31A vs Oh43 2.8.

*Significant at the 95% probability level.

Table 34. Analysis of variance of larval mortality on inbreds WF9, Oh43 and CI31A (greenhouse (Test 2) 1969)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	2	195.3	97.7	4.21*
Error	20	463.8	23.2[a]	
Total	22	659.1		

[a]L.S.D. (.05) = CI31A or Oh43 vs WF9 5.2; CI31A vs Oh43 5.0.

*Significant at the 95% level of probability.

Table 35. Analysis of variance of average weight of surviving larvae on inbreds WF9, Oh43 and CI31A (greenhouse (Test 2) 1969)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	2	0.32	0.16[a]	16.0**
Error	20	0.22	0.01	
Total	22	0.54		

[a]L.S.D. (.05) = CI31A or Oh43 vs WF9 0.20; CI31A vs Oh43 0.20.

**Significant at the 99% level of probability.

Table 36. Greenhouse (test 3) 1970. Extended leaf height, tassel height, tassel bred ratio, number of larvae on traps (daily), number of larvae established, larval mortality and average weight of survivors, on inbreds WF9, Oh43, CI31A, B42, W22 and R101

Inbred	Plant No.	Extended Leaf Height (cm)	Tassel Height (cm)	Tassel Bud Ratio	Number of Larvae on Traps				
					Day 1	Day 2	Day 3	Day 4	Sum
C131A	1	88.9	10.2	11.5	15	6	1	0	22
	2	81.3	10.2	12.6	21	2	1	0	24
	3	91.4	10.2	11.2	4	4	1	7	16
	4	83.8	8.9	10.6	7	4	1	5	17
	5	81.3	6.4	7.9	1	6	0	8	15
	Sum	426.7	45.9	53.7	48	22	4	20	94
	Mean	85.3	9.2	10.7	9.6	4.4	0.8	4.0	18.8
W22	1	81.3	11.4	14.0	5	0	0	0	5
	2	91.4	15.2	16.6	1	2	0	2	5
	3	86.4	11.4	13.2	1	3	0	0	4
	4	85.1	11.4	13.4	0	2	0	5	7
	5	83.8	14.0	16.7	1	5	1	4	11
	Sum	428.0	63.4	74.0	8	12	1	11	32
	Mean	85.6	12.7	14.8	1.6	2.4	0.2	2.2	6.4
Oh43	1	92.7	14.0	15.1	2	2	0	1	5
	2	88.9	11.4	12.8	4	10	1	4	19
	3	91.4	12.7	13.9	3	4	0	0	7
	4	91.4	17.8	19.5	2	1	0	6	9
	5	82.6	6.4	7.8	3	8	0	3	14
	Sum	447.0	62.3	69.0	14	25	1	14	54
	Mean	89.4	12.5	13.8	2.8	5.0	0.2	2.8	10.8
B52	1	83.8	8.9	10.6	0	2	1	0	3
	2	91.4	8.9	9.7	6	0	1	0	7
	3	83.8	6.4	7.6	0	1	2	0	3
	4	88.9	7.6	8.6	0	1	0	1	2
	5	81.3	7.6	9.4	3	2	0	0	5
	Sum	429.2	39.4	45.9	9	6	4	1	20
	Mean	85.8	7.9	9.2	1.8	1.2	0.8	0.2	4.0

Number Larvae Established	Average wt (mg) Survivors	Mortality (number larvae)
2	0.45	6
0	-	6
1	0.15	13
3	0.15	10
4	0.20	11
10	0.95	46
2.0	0.24	9.2
16	0.57	9
15	0.65	10
4	0.38	22
14	0.25	9
8	0.40	11
57	2.25	61
11.4	0.45	12.2
9	1.01	16
7	0.61	4
8	0.45	15
8	0.39	13
12	0.29	4
44	2.75	52
8.8	0.55	10.4
10	0.57	17
10	0.63	13
13	0.43	14
25	0.49	3
14	0.41	11
72	2.53	58
14.4	0.51	11.6

Table 36 (Continued)

Inbred	Plant No.	Extended Leaf Height (cm)	Tassel Height (cm)	Tassel Bud Ratio	Number of Larvae on Traps				
					Day 1	Day 2	Day 3	Day 4	Sum
R101	1	96.4	15.2	15.8	1	0	1	0	2
	2	83.8	12.7	15.2	0	0	0	0	0
	3	86.4	11.4	13.2	1	1	0	0	2
	4	88.9	16.5	18.6	1	0	0	0	1
	5	86.4	11.4	13.2	0	0	1	1	2
	Sum	441.9	67.2	75.9	3	1	2.0	1.0	7.0
	Mean	88.4	13.4	15.2	0.6	0.2	0.4	0.2	1.4
WF9	1	90.2	8.9	9.9	0	0	0	1	1
	2	81.3	14.0	17.2	2	0	1	0	3
	3	88.9	5.1	5.7	3	0	0	0	3
	4	81.3	7.6	9.4	1	2	0	1	4
	5	82.6	3.8	4.6	0	0	1	0	1
	Sum	424.3	39.4	46.8	6	2	2	2	12
	Mean	84.9	7.9	9.4	1.2	0.4	0.4	0.4	2.4

Number Larvae Established	Average wt (mg) Survivors	Mortality (number larvae)
23	1.06	5
28	0.65	2
18	0.76	10
21	0.50	8
27	0.73	1
117	3.70	26
23.4	0.74	5.2
18	0.53	11
24	0.97	3
24	0.99	3
22	0.63	4
21	0.86	8
109	3.98	29
21.8	0.80	5.8

Table 37. Analysis of variance of number of larvae established on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 3) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	1631.8	326.4	20.7**
Error	24	379.2	15.8[a]	
Total	29	2011.0		

[a]L.S.D. (.05) = 5.2.

**Significant at the 99% level of probability.

Table 38. Analysis of variance of number of larvae on traps from inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 3) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	1075.1	215.0	20.7**
Error	24	249.2	10.4[a]	
Total	29	1324.3		

[a]L.S.D. (.05) = 4.2.

**Significant at the 99% level of probability.

Table 39. Analysis of variance of larval mortality on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 3) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	218.3	43.7	2.0[a]
Error	24	523.6	21.8	
Total	29	741.9		

[a]Not significant.

Table 40. Analysis of variance of average weight of surviving larvae on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 3) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	0.94	0.19	4.8**
Error	23	0.85	0.04[a]	
Total	28	1.79		

[a]L.S.D. (.05) = 0.27.

**Significant at the 99% level of probability.

Table 41. Greenhouse (Test 4) 1970. Extended leaf height, tassel height, tassel bud ratio, number of larvae on traps, number of larvae established, larval mortality, and average weight of survivors on inbreds, WF9, Oh43, CI31A, B52, W22 and R101

Inbred	Plant No.	Extended Leaf Height	Tassel Height	Tassel Bud Ratio	Number of Larvae on Traps	Number of Larvae Estab-lished	Average wt (mg) Sur-vivors	Mortality (number larvae)
CI31A	1	96.5	11.4	11.8	11	2	0.50	17
	2	96.5	10.2	10.6	16	1	0.02	13
	3	96.5	12.7	13.2	13	3	0.29	14
	4	96.5	12.7	13.2	12	4	0.50	14
	5	100.3	14.0	14.0	3	3	0.17	24
	Sum	486.3	61.0	62.7	55	13	1.48	82
	Mean	97.3	12.2	12.5	11.0	2.6	0.30	16.4
W22	1	92.7	15.2	16.4	2	8	0.23	20
	2	96.5	15.2	15.8	2	10	0.22	18
	3	101.6	24.1	23.7	0	6	0.19	24
	4	96.5	15.2	15.8	3	13	0.27	14
	5	101.6	43.2	42.5	4	7	0.33	19
	Sum	488.9	112.9	114.1	11	44	1.24	95
	Mean	97.8	22.6	22.8	2.2	8.8	0.25	19
Oh43	1	99.1	20.3	20.5	1	16	0.42	13
	2	99.1	34.3	34.6	8	9	0.61	13
	3	106.7	17.8	16.7	3	21	0.47	6
	4	100.3	12.7	12.7	5	12	0.44	13
	5	91.4	15.2	16.6	0	11	0.48	19
	Sum	496.6	100.3	101.1	17	69	2.42	64
	Mean	99.3	20.1	20.2	3.4	13.8	0.48	12.8
B52	1	94.0	10.2	10.9	3	2	0.65	25
	2	101.6	19.1	18.8	5	5	0.45	20
	3	96.5	11.4	11.8	0	14	0.46	16
	4	94.0	12.7	13.5	3	11	0.45	16
	5	96.5	12.7	13.2	1	10	0.39	19
	Sum	482.6	66.1	68.1	12	42	2.40	96
	Mean	96.5	13.2	13.6	2.4	8.4	0.48	19.2

Table 41 (Continued)

Inbred	Plant No.	Extended Leaf Height	Tassel Height	Tassel Bud Ratio	Number of Larvae on Traps	Number of Larvae Estab-lished	Average wt (mg) Sur-vivors	Mortality (number larvae)
R101	1	91.4	14.0	15.3	0	11	0.70	19
	2	90.2	12.7	14.1	0	8	0.58	22
	3	99.1	22.9	23.1	0	15	0.67	15
	4	88.9	10.2	11.5	0	12	0.48	18
	5	100.3	35.6	35.5	0	16	0.63	14
	Sum	469.9	95.4	99.5	0	62	3.06	88
	Mean	94.0	19.1	19.9	0	12.4	0.61	17.6
WF9	1	95.3	11.4	12.0	0	25	0.57	5
	2	90.2	6.4	7.1	0	23	0.79	7
	3	94.0	12.7	13.5	1	19	0.40	10
	4	96.5	16.5	17.1	1	20	0.66	9
	5	94.0	14.0	14.9	0	20	0.48	10
	Sum	470.0	61.0	64.6	2	107	2.90	41
	Mean	94.0	12.2	12.9	0.4	21.4	0.58	8.2

Table 42. Analysis of variance of number of larvae established on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 4) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	999.0	199.8	16.8**
Error	24	286.4	11.9[a]	
Total	29	1285.4		

[a] L.S.D. (.05) = 4.5.

** Significant at the 99% level of probability.

Table 43. Analysis of variance of number of larvae on traps from inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 4) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	403.0	80.6	12.0**
Error	24	160.4	6.7[a]	
Total	29	563.4		

[a]L.S.D. (.05) = 3.4.

**Significant at the 99% level of probability.

Table 44. Analysis of variance of larval mortality on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 4) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	440.7	88.1	7.2**
Error	24	292.0	12.2[a]	
Total	29	732.7		

[a]L.S.D. (.05) = 4.5.

**Significant at the 99% level of probability.

Table 45. Analysis of variance of average weight of surviving larvae on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 4) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	0.55	0.11	5.5**
Error	24	0.37	0.02[a]	
Total	29	0.92		

[a]L.S.D. (.05) = 0.17.

**Significant at the 99% level of probability.

Table 46. Greenhouse (Test 5) 1970. Extended leaf height, tassel height, tassel bud ratio, number of larvae on traps, number of larvae established, larval mortality and average weight of survivors on inbreds, WF9, Oh43, CI31A, B52, W22 and R101

Inbred	Plant No.	Extended Leaf Height	Tassel Height	Tassel Bud Ratio	Number of Larvae on Traps	Number of Larvae Estab-lished	Average wt (mg) Sur-vivors	Mortality (number larvae)
CI31A	1	82.6	6.4	7.8	9	3	0.35	18
	2	92.7	7.6	8.2	13	1	0.65	16
	3	96.5	12.7	13.2	12	4	0.49	14
	4	91.4	17.8	19.5	16	1	0.32	13
	5	86.4	6.4	7.4	11	0	-	19
	Sum	449.6	50.9	56.0	61	9	1.81	80
	Mean	89.9	10.2	11.2	12.2	1.8	0.45	16.0
W22	1	96.5	20.3	21.0	0	1	0.61	29
	2	96.5	12.7	13.2	2	13	0.67	15
	3	95.3	16.5	17.3	4	4	0.53	22
	4	87.6	8.9	10.2	0	14	0.25	16
	5	90.2	11.4	12.6	15	3	0.31	12
	Sum	466.1	69.8	74.3	21	35	2.37	94
	Mean	93.2	14.0	14.9	4.2	7.0	0.47	18.8
Oh43	1	105.4	10.2	9.7	2	17	0.94	11
	2	106.7	10.2	9.6	2	2	0.64	26
	3	101.6	8.9	8.8	0	12	0.55	18
	4	100.3	17.8	17.8	2	16	0.71	12
	5	76.2	10.2	13.4	12	8	0.33	10
	Sum	490.2	57.3	59.1	18	55	3.17	77
	Mean	98.0	11.5	11.8	3.6	11	0.63	15.4
B52	1	95.3	14.0	14.7	0	25	0.49	5
	2	99.1	14.0	14.1	1	6	0.64	23
	3	106.7	15.2	14.3	5	26	0.74	0
	4	92.7	8.9	9.6	3	13	0.30	14
	5	87.6	5.1	5.8	0	9	0.56	21
	Sum	481.4	57.2	58.5	9	79	2.73	63
	Mean	96.3	11.4	11.7	1.8	15.8	0.55	12.6

Table 46 (Continued)

Inbred	Plant No.	Extended Leaf Height	Tassel Height	Tassel Bud Ratio	Number of Larvae on Traps	Number of Larvae Estab-lished	Average wt (mg) Sur-vivors	Mortality (number larvae)
R101	1	92.7	8.8	9.6	2	5	0.99	23
	2	94.0	15.2	16.2	0	18	0.67	12
	3	95.3	12.7	13.3	0	21	0.75	9
	Sum	282.0	36.8	39.1	2	44	2.41	44
	Mean	94.0	12.3	13.0	0.7	14.7	0.80	14.6
WF9	1	108.0	19.1	17.7	0	13	1.02	17
	2	110.5	22.9	20.7	0	14	0.57	16
	3	105.4	15.2	14.4	0	5	0.72	25
	4	101.6	11.4	11.2	1	22	0.45	7
	Sum	425.5	68.6	64.1	1	54	2.76	65
	Mean	106.4	17.2	16.0	0.3	13.5	0.67	16.2

Table 47. Analysis of variance of number of larvae established on inbreds WF9, CI31A, W22 and R101 (greenhouse (Test 5) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	667.4	133.5	3.0*
Error	21	937.3	44.6[a]	
Total	26	1604.7		

[a]L.S.D. (.05) = WF9 vs R101 10.6; rest vs R101 10.2; rest vs WF9 9.4; between rest 8.7.

*Significant at the 95% level of probability.

Table 48. Analysis of variance of number of larvae on traps from inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 5) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	450.4	90.1	6.3**
Error	21	297.2	14.2[a]	
Total	26	747.6		

[a]L.S.D. (.05) = WF9 vs R101 6.0; rest vs R101 5.8; rest vs WF9 5.2; between rest 5.0.

**Significant at the 99% level of probability.

Table 49. Analysis of variance of larval mortality on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 5) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	101.4	20.3	<1[a]
Error	21	1056.6	50.3	
Total	26	1158.0		

[a]Not significant.

Table 50. Analysis of variance of average weight of surviving larvae on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (greenhouse (Test 5) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	0.38	0.08	2.0[a]
Error	20	0.76	0.04	
Total	25	1.14		

[a]Not significant.

Table 51. Field (Test 6) 1971. Extended leaf height, tassel height, tassel bud ratio, number of larvae on traps, number of larvae established and larval mortality on inbreds WF9, OH43, CI31A, B52, W22 and R101

Inbred	Plant No.	Extended Leaf Height (cm)	Tassel Height (cm)	Tassel Bud Ratio	Number of Larvae on Traps	Number of Larvae Estab-lished	Mortality (number larvae)
CI31A	1	66.0	3.8	5.8	17	2	11
	2	76.2	5.1	6.7	10	1	19
	3	88.9	7.6	8.5	10	4	16
	Sum	230.9	16.5	21.0	37	7	46
	Mean	77.0	5.5	7.0	12.33	2.33	15.33
W22	1	76.2	7.6	10.0	12	7	11
	2	81.3	6.4	7.9	4	6	20
	3	94.0	11.4	12.1	4	7	19
	Sum	251.5	25.4	30.0	20	20	50
	Mean	83.8	8.5	10.0	6.67	6.67	16.67
Oh43	1	83.8	10.2	12.2	6	2	22
	2	76.2	7.6	10.0	2	7	21
	3	88.9	12.7	14.3	1	7	22
	Sum	248.9	30.5	36.5	9	16	65
	Mean	83.0	10.2	12.2	3.0	5.33	21.67
B52	1	76.2	5.7	7.5	8	3	19
	2	81.3	6.4	7.9	1	9	20
	3	94.0	12.7	13.5	1	4	25
	Sum	251.5	24.8	28.9	10	16	64
	Mean	83.8	8.3	9.6	3.33	5.33	21.33
R101	1	68.6	5.7	8.3	6	10	14
	2	83.8	6.4	7.6	1	9	20
	3	83.8	10.2	12.2	4	7	19
	Sum	236.2	22.3	28.1	11	26	53
	Mean	78.7	7.4	9.4	3.67	8.67	17.67
WF9	1	81.3	5.7	7.0	6	10	14
	2	88.9	7.6	8.5	2	10	18
	3	94.0	10.2	10.9	1	7	22
	Sum	264.2	23.5	26.4	9	27	54
	Mean	88.1	7.8	8.8	3.0	9.0	18.0

Table 52. Analysis of variance of number of larvae established on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (field (Test 6) 1971)

Source of Variation	d.f.	S.S.	M.S.	F
Blocks	2	5.8	2.9	<1[a]
Inbreds	5	91.8	18.4[b]	3.8*
Error	10	47.5	4.8	
Total	17	145.1		

[a]Not significant.

[b]L.S.D. (.05) = 4.0.

*Significant at the 95% level of probability.

Table 53. Analysis of variance of number of larvae on traps from inbreds WF9, Oh43, CI31A, B52, W22 and R101 (field (Test 6), 1971)

Source of Variation	d.f.	S.S.	M.S.	F
Blocks	2	132.3	66.2	41.4**
Inbreds	5	205.3	41.1[a]	27.7**
Error	10	16.4	1.6	
Total	17	354.0		

[a]L.S.D. (.05) = 2.3.

**Significant at the 99% level of probability.

Table 54. Analysis of variance of larval mortality on inbreds WF9, Oh43, CI31A, B52, W22 and R101 (field (Test 6) 1970)

Source of Variation	d.f.	S.S.	M.S.	F
Blocks	2	98.8	49.4	8.7**
Inbreds	5	97.1	19.4	3.4*
Error	10	56.5	5.7[a]	
Total	17	252.4		

[a]L.S.D. (.05) = 4.3.

*Significant at the 95% level of probability.

**Significant at the 99% level of probability.

Table 55. Combined analysis of variance of the number of larvae trapped

Source of Variation	d.f.	S.S.	M.S.	F
Runs[a]	2	62.81	31.41	8.73**
Inbreds	5	360.79	72.16	20.04**
Error[b]	10	35.96	3.60	
Total	17	459.56		

[a]Means from individual tests (3-5).

[b]L.S.D. (.01) = 4.91.

**Significant at the 99% level of probability.

Table 56. Combined analysis of variance of the number of larvae established

Source of Variation	d.f.	S.S.	M.S.	F
Runs[a]	2	28.39	14.20	1.04[b]
Inbreds	5	533.62	106.72	7.82**
Error[c]	10	136.46	13.65	
Total	17	698.47		

[a]Means from individual tests (3-5).

[b]Not significant.

[c]L.S.D. (.01) = 9.57.

**Significant at the 99% level of probability.

Table 57. Combined analysis of variance of the average weight of surviving larvae

Source of Variation	d.f.	S.S.	M.S.	F
Runs[a]	2	0.08	0.04	8.00**
Inbreds	5	0.37	0.07	14.00**
Error[b]	10	0.05	0.005	
Total	17			

[a]Means from individual tests (3-5).

[b]L.S.D. (.01) = 0.13.

**Significant at the 99% level of probability.

Table 58. Combined analysis of variance of the larval mortality on the plant

Source of Variation	d.f.	S.S.	M.S.	F
Runs[a]	2	173.99	86.99	10.02**
Inbreds	5	71.57	14.30	1.64[b]
Error	10	86.82	8.68	
Total	17	332.38		

[a]Means from individual test (3-5).

[b]Not significant.

**Significant at the 99% level of probability.

Table 59. HBOA concentration for six inbred lines of corn (greenhouse test)[a]

Inbred	Replicate			Mean
	I	II	III[b]	
WF9	2.55	1.70	0.97	1.74
R101	3.27	3.76	0.91	2.65
W22	3.88	4.61	0.91	3.13
B52	2.67	3.03	0.85	2.18
Oh43	3.03	3.09	0.85	2.32
CI31A	3.52	3.88	0.85	2.75

[a]Concentration expressed as μ mol/g dry weight tissue.

[b]Spectrophotometric analysis.

Table 60. Analysis of variance of HBOA concentration, greenhouse test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	2	22.37	11.19	41.44**
Inbred	5	3.55	0.71	2.62[a]
Error	10	2.68	0.27	
Total	17	28.60		

[a]Not significant.

**Significant at the 99% level of probability.

Table 61. HMBOA concentration for six inbred lines of corn (greenhouse test)[a]

Inbred	Replicate			Mean
	I	II	III	
WF9	0.82	1.28	0.56	0.89
R101	0.87	1.85	0.77	1.16
W22	1.08	3.28	1.13	1.83
B52	0.87	1.95	0.92	1.25
Oh43	0.87	1.54	1.03	1.15
CI31A	1.54	4.67	3.03	3.08

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 62. Analysis of variance of HMBOA concentration, greenhouse test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	2	6.96	3.48	11.22**
Inbred	5	9.78	1.95	6.29**
Error	10	3.07	0.31	
Total	17	19.81		

**Significant at the 99% level of probability.

Table 63. DIBOA concentration for six inbred lines of corn (grenhouse test)[a]

Inbred	Replicate			Mean
	I	II	III	
WF9	2.21	4.53	2.82	3.19
R101	3.92	5.47	2.60	4.00
W22	2.10	4.59	3.20	3.30
B52	2.38	3.87	3.43	3.23
Oh43	1.77	6.74	3.31	3.94
CI31A	6.13	5.08	3.43	4.88

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 64. Analysis of variance of DIBOA concentration, greenhouse test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	2	15.03	7.51	5.82*
Inbred	5	6.51	1.30	1.00[a]
Error	10	12.92	1.29	
Total	17	34.46		

[a]Not significant.

*Significant at the 95% level of probability.

Table 65. DIMBOA concentration for six inbred lines of corn (greenhouse test)[a]

Inbred	Replicate			Mean
	I	II	III	
WF9	7.30	6.16	5.82	6.43
R101	12.65	10.00	7.53	10.06
W22	26.16	16.59	15.01	19.25
B52	12.65	8.48	8.79	9.97
Oh43	19.38	11.71	9.58	13.56
CI31A	34.36	21.71	21.53	25.77

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 66. Analysis of variance of DIMBOA concentration, greenhouse test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	2	192.29	96.14	13.81**
Inbred	5	765.48	153.10	22.00**
Error	10	69.64	6.96	
Total	17	1027.41		

**Significant at the 99% level of probability.

Table 67. DIM_2BOA concentration for six inbred lines of corn (greenhouse test)[a]

Inbred	Replicate			Mean
	I	II	III	
WF9	5.44	7.22	3.07	5.24
R101	4.19	10.46	5.85	6.83
W22	3.86	7.34	2.66	4.62
B52	3.61	5.85	3.49	4.32
Oh43	2.12	6.76	2.86	3.91
CI31A	5.15	4.52	2.99	4.22

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 68. Analysis of variance of DIM_2BOA concentration, greenhouse test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	2	43.66	21.63	13.18**
Inbred	5	17.09	3.41	2.07[a]
Error	10	16.37	1.64	
Total	17	77.12		

[a]Not significant.

**Significant at the 99% level of probability.

Table 69. HBOA concentration for six inbred lines of corn (field test)[a]

Inbred	Replicate		Mean
	I	II	
WF9	2.61	5.70	4.16
R101	3.27	5.33	4.30
W22	3.15	7.52	5.34
B52	2.96	3.94	3.45
Oh43	3.33	6.73	5.03
CI31A	4.30	3.58	3.94

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 70. Analysis of variance of HBOA concentration, field test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	1	14.48	14.48	8.52*
Inbred	5	4.91	0.98	<1[a]
Error	5	8.58	1.70	
Total	11	27.87		

[a]Not significant.

*Significant at the 95% level of probability.

Table 71. HMBOA concentration for six inbred lines of corn (field test)[a]

Inbred	Replicate I	Replicate II	Mean
WF9	1.02	1.70	1.36
R101	1.69	3.04	2.37
W22	1.76	2.07	1.92
B52	1.73	2.30	2.02
Oh43	1.52	2.39	1.96
CI31A	3.76	3.13	3.45

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 72. Analysis of variance of HMBOA concentration, field test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	1	0.83	0.83	3.77[a]
Inbred	5	4.91	0.98	4.46[a]
Error	5	1.10	0.22	
Total	11	6.84		

[a]Not significant.

Table 73. DIBOA concentration for six inbred lines of corn (field test)[a]

Inbred	Replicate I	Replicate II	Mean
WF9	2.65	3.93	3.29
R101	4.92	4.74	4.83
W22	4.70	-	4.70
B52	4.92	4.74	4.83
Oh43	5.75	3.48	4.62
CI31A	3.26	4.96	4.11

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 74. Analysis of variance of DIBOA concentration, field test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	1	0.01	0.01	<1[a]
Inbred	5	3.68	0.75	<1[a]
Error	4	4.87	1.22	
Total	10	8.56		

[a]Not significant.

Table 75. DIMBOA concentration for six inbred lines of corn (field test)[a]

Inbred	Replicate		Mean
	I	II	
WF9	7.11	5.69	6.40
R101	15.64	9.62	12.63
W22	16.40	10.47	13.44
B52	11.37	8.86	10.12
Oh43	13.36	12.09	12.73
CI31A	25.45	20.57	23.01

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 76. Analysis of variance of DIMBOA concentration, field test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	1	40.45	40.45	16.71**
Inbred	5	304.94	60.99	25.20**
Error	5	12.12	2.42	
Total	11	357.51		

**Significant at the 99% level of probability.

Table 77. DIM_2BOA concentration for six inbred lines of corn (field test)[a]

Inbred	Replicate		Mean
	I	II	
WF9	5.02	5.06	5.04
R101	6.56	6.68	6.62
W22	5.44	6.51	5.98
B52	5.44	3.20	4.32
Oh43	4.52	7.63	6.08
CI31A	4.81	2.99	3.90

[a]Concentration expressed as μ mol/g dry weight tissue.

Table 78. Analysis of variance of DIM_2BOA concentration, field test

Source of Variation	d.f.	S.S.	M.S.	F
Rep	1	0.01	0.01	<1[a]
Inbred	5	11.57	2.31	1.21[a]
Error	5	9.58	1.92	
Total	11	21.15		

[a]Not significant.

Total 79. HMBOA concentrations for six inbred lines of corn, determined by spectrophotometric analysis[a]

Inbred	Replicate			Mean
	I	II	III	
WF9	0.72	0.72	0.97	0.80
R101	0.97	0.87	0.77	0.87
W22	0.92	1.03	1.38	1.11
B52	0.97	0.82	0.82	0.87
Oh43	0.82	0.82	0.87	0.84
CI31A	1.49	1.79	1.38	1.55

[a]Plant tissue from greenhouse test one; HMBOA concentrations expressed as μ mol HMBOA/g dry weight tissue.

Table 80. Analysis of variance of HMBOA concentrations, determined by spectrophotometric analysis

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds[a]	5	1.25	0.25	8.33**
Error[b]	12	0.28	0.03	
Total	17	1.53		

[a]Tissue from greenhouse test one.

[b]Standard deviation = ± 0.17.

**Significant at the 99% level of probability.

Table 81. HBOA concentration for six inbred lines of corn, determined by spectrophotometric analysis[a]

Inbred	Replicate		Mean
	I	II	
WF9	0.97	0.96	0.97
R101	1.01	0.82	0.97
W22	0.90	0.88	0.89
B52	1.01	0.71	0.86
Oh43	0.82	0.82	0.82
CI31A	0.87	0.82	0.85

[a]HBOA concentration expressed as μ mol HBOA/g dry weight tissue; tissue samples from greenhouse test three.

Table 82. Analysis of variance of HBOA concentrations of six inbred lines of corn, determined by spectrophotometric analysis

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds[a]	5	0.027	0.005	<1[b]
Error[c]	6	0.064	0.011	
Total	11	0.091		

[a]Tissue from greenhouse test three.

[b]Not significant.

[c]Standard deviation = ± 0.105.

Table 83. Standard error of an inbred mean for HBOA, HMBOA, DIBOA, DIMBOA and DIM_2BOA

	1,4-benzoxazin-3-ones				
	HBOA	HMBOA	DIBOA	DIMBOA	DIM_2BOA
Standard error of the mean[a]	0.30	0.32	0.66	1.52	0.74

[a]Expressed as μ mol/g dry tissue.

Table 84. MBOA concentration of six inbred lines of corn, determined by spectrophotometric analysis[a]

Inbred	Replicate		Mean
	I	II	
WF9	0.79	0.75	0.77
R101	0.94	1.14	1.04
W22	2.05	2.13	2.09
B52	1.16	1.03	1.10
Oh43	1.16	1.13	1.15
CI31A	3.24	2.77	3.02

[a]MBOA concentration expressed as mg MBOA/g dry weight tissue.

Table 85. Analysis of variance of MBOA concentrations of six inbred lines of corn, determined by spectrophotometric analysis

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	7.29	1.46	73.00**
Error	6	0.14	0.02	
Total	11	7.43		

**Significant at the 99% level of probability.

Table 86. MBOA concentration of six inbred lines of corn, determined by isotope dilution analysis[a]

Inbred	Replicate		Mean
	I	II	
WF9	1.11	0.92	1.02
R101	1.23	1.34	1.29
W22	2.57	2.50	2.54
B52	1.67	1.33	1.50
Oh43	1.61	1.77	1.69
CI31A	3.46	3.55	3.51

[a]MBOA concentration expressed as mg MBOA/g dry weight tissue.

Table 87. Analysis of variance of MBOA concentrations for six inbred lines of corn, determined by isotope dilution analysis

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	8.69	1.74	87.00**
Error	6	0.10	0.02	
Total	11	8.79		

**Significant at the 99% level of probability.

Table 88. Plate rating values for six inbred lines of corn

Inbred	Observer	Replicate I Plate Rating	Replicate II Plate Rating
WF9	1	1	1
	2	1	1
	3	1	1
R101	1	2	1
	2	2	1
	3	2	1
W22	1	4	3
	2	3	2
	3	4	2
B52	1	2	2
	2	2	1
	3	2	1
Oh43	1	2	2
	2	3	1
	3	3	1
CI31A	1	4	4
	2	5	4
	3	4	4

Table 89. Analysis of variance of average plate rating values

Source of Variation	d.f.	S.S.	M.S.	F
Inbreds	5	13.51	2.70	6.28*
Error	6	2.57	0.43	
Total	11	16.08		

*Significant at the 95% level of probability.

Table 90. Confinement test

Level[a]	Average	Sex	Replicates I	II	III	IV	V	Mean
25.28	Days to pupation	Male	19.71	20.36	20.57	19.40	20.83	20.17
		Female	22.30	23.00	22.11	22.55	21.90	22.37
	Pupal weights[b]	Male	63.30	64.61	67.97	73.68	62.68	66.45
		Female	86.02	93.67	89.10	90.57	86.33	89.14
12.64	Days to pupation	Male	19.00	18.67	17.33	17.40	18.64	18.21
		Female	19.44	19.75	19.33	20.18	19.75	19.69
	Pupal weights	Male	70.84	69.73	65.93	77.34	67.48	70.26
		Female	93.51	97.38	92.49	94.06	103.98	96.28
6.32	Days to pupation	Male	17.38	17.40	18.00	17.50	17.80	17.62
		Female	18.86	18.40	18.50	18.63	18.83	18.64
	Pupal weights	Male	69.66	73.75	77.60	74.06	66.86	72.39
		Female	96.71	97.72	97.42	97.59	96.05	97.10
3.16	Days to pupation	Male	16.71	16.73	17.40	17.50	17.00	17.07
		Female	19.11	17.83	18.83	19.00	17.90	18.53
	Pupal weights	Male	78.86	75.95	79.42	75.18	73.84	76.65
		Female	101.74	108.02	97.22	108.53	101.60	103.42
1.58	Days to pupation	Male	16.56	16.67	17.25	17.57	16.89	16.99
		Female	18.00	18.14	17.89	18.00	18.20	18.05
	Pupal weights	Male	78.06	78.86	72.73	77.10	71.93	75.86
		Female	98.69	106.39	103.51	108.09	100.16	103.37
Control	Days to pupation	Male	16.75	17.50	17.08	17.38	17.60	17.26
		Female	17.75	17.14	17.33	18.44	18.00	17.73
	Pupal weights	Male	76.33	76.95	76.23	74.85	77.92	76.46
		Female	102.21	111.66	103.67	99.06	104.32	104.18

[a]Level = μ mol DIMBOA/g dry weight diet.

[b]Weight expressed in mg.

Table 91. Analysis of variance of average female pupal weight (mg)

Source of Variation	d.f.	M.S.	F
Blocks	4	32.98	2.75*
Level of DIMBOA	5	173.72	14.50**
Error	20	11.98[a]	
Total	29		

[a]L.S.D. (.01) = 6.22.

*Significant at 95% level of probability.

**Significant at 99% level of probability.

Table 92. Analysis of variance of average male pupal weight (mg)

Source of Variation	d.f.	M.S.	F
Blocks	4	21.16	1.95[a]
Level of DIMBOA	5	84.26	7.75**
Error[b]	20	10.87	
Total	29		

[a]Not significant.

[b]L.S.D. (.01) = 5.93).

**Significant at the 99% level of probability.

Table 93. Analysis of variance of average days to pupation for males

Source of Variation	d.f.	M.S.	F
Blocks	4	0.17	0.63[a]
Level of DIMBOA	5	7.28	27.73**
Error[b]	20	0.26	
Total	29		

[a]Not significant.

[b]L.S.D. (.01) = 0.91.

**Significant at the 99% level of probability.

Table 94. Analysis of variance of average days to pupation for females

Source of Variation	d.f.	M.S.	F
Blocks	4	0.22	1.38[a]
Level of DIMBOA	5	14.54	92.64**
Error[b]	20	0.16	
Total	29		

[a]Not significant.

[b]L.S.D. (.01) = 0.71.

**Significant at 99% level of probability.

Table 95. Choice test with one day old larvae[a]

Replicate	Time of Observation (hrs)							
	24		48		72		96	
	Level							
	Control	12.64	Control	12.64	Control	12.64	Control	12.64
1	7	0	5	1	8	0	5	0
2	7	0	6	1	5	1	6	1
3	3	5	5	5	5	3	4	1
4	5	1	5	0	5	1	3	1
5	5	2	5	3	4	4	3	2
6	6	2	6	2	6	2	4	4
7	7	1	8	1	4	1	4	1
8	6	4	6	1	6	1	4	0
9	6	3	6	2	4	2	4	2
10	4	1	6	1	4	1	2	1
11	5	1	7	1	2	3	3	4
12	5	3	4	1	6	1	5	1
13	6	2	6	2	5	3	2	3
14	3	2	4	3	4	3	5	2
15	7	1	6	2	6	2	4	4
16	7	1	6	1	5	1	6	1
17	4	3	5	2	3	3	3	2
18	4	3	3	4	2	6	3	2
19	5	4	3	3	5	3	3	1
20	5	2	3	0	5	0	4	1
21	6	1	7	1	8	2	4	0
22	4	2	5	4	4	3	3	3
23	7	1	6	1	4	2	4	4
24	7	2	7	2	5	2	5	2
25	8	1	8	1	6	1	3	3
Sum	139	48	138	45	121	51	96	46
Mean	5.56	1.92	5.52	1.80	4.84	2.04	3.84	1.84
t(48 df)	9.73**		9.94**		7.00**		6.02**	

[a]Data recorded are larval counts.

**Significant at 99% level of probability.

Table 96. Choice test with five day old larvae[a]

Replicate	Time of Observation (hrs)							
	24		48		72		96	
	Level							
	Control	12.64	Control	12.64	Control	12.64	Control	12.64
1	1	1	2	1	5	0	2	2
2	2	2	2	2	1	1	1	2
3	0	1	1	1	1	1	2	1
4	2	0	3	0	3	1	4	1
5	1	2	1	2	1	1	1	2
6	0	0	3	0	2	1	2	1
7	1	3	2	1	1	1	1	1
8	2	2	3	0	2	1	1	2
9	4	1	1	1	3	1	3	1
10	2	0	3	0	1	3	1	1
11	1	1	1	2	1	2	2	2
12	2	1	2	1	2	0	1	1
13	1	0	1	3	1	4	2	2
14	1	1	1	2	3	0	3	1
15	2	0	0	1	2	1	1	1
16	1	0	2	2	2	0	4	1
17	2	0	1	1	0	1	2	0
18	1	2	3	1	4	1	3	0
19	2	1	1	0	1	1	2	2
20	3	0	1	0	1	1	1	1
21	1	2	3	0	3	0	3	1
22	2	1	1	0	2	1	3	0
23	1	1	1	0	5	0	2	1
24	3	2	2	2	2	0	2	1
25	2	2	1	1	2	0	2	2
26	3	1	3	1	2	0	2	2
27	3	1	0	2	3	0	1	2
28	2	1	2	1	2	1	2	1
29	0	1	3	1	2	1	2	2
30	2	1	1	1	4	0	2	1
Sum	50	31	51	30	64	25	59	39
Mean	1.66	1.03	1.70	1.00	2.13	0.83	1.97	1.30
t(58 df)	2.77**		3.03**		4.66**		3.31**	

[a]Data recorded are larval counts.

**Significant at 99% level of probability.

Table 97. Choice test with ten day old larvae[a]

Replicate	Time of Observation (hrs)							
	24		48		72		96	
	Level							
	Control	12.64	Control	12.64	Control	12.64	Control	12.64
1	2	0	3	2	2	1	0	0
2	1	1	0	1	2	1	0	0
3	1	1	2	0	1	0	1	1
4	2	1	1	3	2	1	2	1
5	1	0	1	1	0	2	2	0
6	1	0	3	1	2	0	1	1
7	2	1	2	1	2	1	3	1
8	3	0	2	0	3	0	4	1
9	1	1	1	1	2	1	2	0
10	2	1	0	0	2	0	0	0
11	3	1	3	0	0	0	2	0
12	2	0	1	0	1	1	0	1
13	1	0	2	1	1	0	1	0
14	1	0	1	1	2	1	1	0
15	2	2	0	1	0	1	1	1
16	0	1	2	1	1	1	2	1
17	1	0	1	2	1	1	0	1
18	3	0	2	2	0	0	1	0
19	1	0	2	0	0	1	0	0
20	0	1	1	1	2	0	1	0
21	1	1	3	1	2	0	0	1
22	0	0	0	1	2	1	1	0
23	2	1	1	1	1	1	1	2
24	2	0	0	1	2	0	1	0
25	1	2	2	0	1	0	1	0
26	0	1	3	1	2	2	2	1
27	2	1	1	0	1	1	0	1
28	1	0	2	1	3	0	1	2
29	0	2	3	0	1	0	1	0
30	2	0	2	0	3	0	3	0
Sum	41	19	47	25	44	18	36	17
Mean	1.37	0.63	1.57	0.83	1.47	0.60	1.20	0.57
t(58 df)	3.61**		3.21**		4.34**		3.10**	

[a]Data recorded are larval counts.

**Significant at 99% level of probability.

Table 98. Choice tests[a]

Level[b]	Petri Dish	Run I Location: Control Diet	Treated Diet	Dish Surface	Total
0.39	1	6	2	1	9
	2	3	5	1	9
	3	3	5	2	10
	4	2	7	1	10
	5	4	3	0	7
	Total	18	22	5	45
0.79	1	6	3	0	9
	2	8	2	0	10
	3	4	3	1	7
	4	5	3	0	8
	5	4	5	1	10
	Total	27	16	2	45
1.58	1	2	2	2	6
	2	5	2	2	9
	3	6	0	2	8
	4	3	5	0	8
	5	7	2	0	9
	Total	23	11	6	40
3.16	1	4	4	0	8
	2	6	3	1	10
	3	6	3	0	9
	4	10	0	0	10
	5	2	8	0	10
	Total	28	18	1	47
6.32	1	5	1	3	9
	2	5	2	0	7
	3	4	4	0	8
	4	4	3	0	7
	5	6	3	1	10
	Total	24	16	4	44
12.64	1	6	3	0	9
	2	6	2	1	9
	3	5	2	2	9
	4	7	2	0	9
	5	5	4	1	10
	Total	29	13	4	46

[a]Data recorded are larval counts.

[b]Level expressed as u mol DIMBOA/g diet; each level represents a separate choice test designed as a split plot with runs being the whole plot and control vs treated being the split plot.

Run II				Run III			
Location				Location			
Control Diet	Treated Diet	Dish Surface	Total	Control Diet	Treated Diet	Dish Surface	Total
3	5	1	9	5	3	1	9
5	3	1	9	5	5	0	10
3	4	2	9	4	3	1	8
3	6	1	10	4	6	0	10
6	3	0	9	4	0	0	4
20	21	5	46	22	17	2	41
3	3	0	6	5	3	0	8
4	4	0	8	4	2	1	7
3	6	0	9	4	5	1	10
4	6	0	10	3	3	0	6
4	2	1	7	3	5	1	9
18	21	1	40	19	18	3	40
3	7	0	10	5	6	0	11
5	3	2	10	5	5	0	10
4	6	0	10	4	6	0	10
3	2	0	5	6	3	1	10
4	4	3	11	4	3	3	10
19	22	5	46	24	23	4	51
8	3	0	11	5	1	1	7
5	4	1	10	7	2	1	10
4	6	0	10	1	5	0	6
9	1	0	10	6	3	0	9
8	1	0	9	3	5	0	8
34	15	1	50	22	16	2	40
6	4	0	10	6	1	0	7
6	3	0	9	5	2	0	7
1	6	0	7	2	3	1	6
6	3	1	10	4	5	1	10
7	2	0	9	7	2	0	9
26	18	1	45	24	13	2	39
5	4	1	10	3	3	0	6
3	3	2	8	3	6	0	9
7	3	0	10	4	5	0	9
6	2	2	8	6	1	0	7
5	4	2	11	7	1	2	10
26	16	7	49	23	16	2	43

Table 98 (Continued)

Level[b]	Petri Dish	Run I Location: Control Diet	Treated Diet	Dish Surface	Total
25.28	1	4	2	0	6
	2	5	0	3	8
	3	7	1	1	9
	4	7	2	1	10
	5	5	2	4	11
	Total	28	10	6	44

Run II				Run III			
Location				Location			
Control Diet	Treated Diet	Dish Surface	Total	Control Diet	Treated Diet	Dish Surface	Total
3	6	1	10	5	4	1	10
8	2	0	10	6	2	1	9
8	2	0	10	5	3	1	9
5	5	0	10	9	1	0	10
5	5	0	10	6	3	1	10
29	20	1	50	31	13	4	48

Table 99. Analysis of variance of choice test, 0.39 μ mol DIMBOA/g dry weight diet

Source of Variation	d.f.	S.S.[a]	M.S.	F
Reps	4	0.47	0.12	1.20[b]
Run	2	0.04	0.02	-[b]
Error (a)	8	0.77	0.10	
Level	1	0.06	0.06	-[b]
Level x Run	2	1.03	0.52	2.74[b]
Error (b)	12	2.22	0.19	
Total	29	4.59		

[a]Larval counts transformed to $\sqrt{X + .05}$.

[b]Not significant.

Table 100. Analysis of variance of choice test, 0.79 μ mol DIMBOA/g dry weight diet

Source of Variation	d.f.	S.S.[a]	M.S.	F
Reps	4	0.03	0.008	-[b]
Run	2	0.08	0.04	-[b]
Error (a)	8	0.63	0.08	
Level	1	0.17	0.17	1.70[b]
Level x Run	2	0.75	0.38	3.80[b]
Error (b)	12	1.20	0.10	
Total	29	2.86		

[a]Larval counts transformed to $\sqrt{X + 0.5}$.

[b]Not significant.

Table 101. Analysis of variance of choice test, 1.58 μ mol DIMBOA/g dry weight diet

Source of Variation	d.f.	S.S.[a]	M.S.	F
Reps	4	0.05	0.013	-[b]
Run	2	0.73	0.37	2.43[b]
Error (a)	8	1.23	0.15	
Level	1	0.31	0.31	1.35[b]
Level x Run	2	0.80	0.40	1.74[b]
Error (b)	12	2.61	0.23	
Total	29	5.73		

[a]Larval counts transformed to $\sqrt{X + .05}$.

[b]Not significant.

Table 102. Analysis of variance of choice test, 3.16 μ mol DIMBOA/g dry weight diet

Source of Variation	d.f.	S.S.[a]	M.S.	F
Reps	4	0.04	0.01	-[b]
Run	2	0.27	0.14	2.00[b]
Error (a)	8	0.54	0.07	
Level	1	2.29	2.29	5.20*
Level x Run	2	3.03	1.51	3.43[b]
Error (b)	12	5.30	0.44	
Total	29	11.47		

[a]Larval counts transformedto $\sqrt{X + 0.5}$.

[b]Not significant.

*Significant at the 95% level of probability.

Table 103. Analysis of variance of choice test, 6.32 µ mol DIMBOA/g dry weight diet

Source of Variation	d.f.	S.S.[a]	M.S.	F
Reps	4	0.28	0.07	-[b]
Run	2	0.16	0.08	-[b]
Error (a)	8	0.49	0.06	
Level	1	1.69	1.69	6.04*
Level x Run	2	0.09	0.05	-[b]
Error (b)	12	3.35	0.28	
Total	29	6.06		

[a]Larval counts transformed to $\sqrt{X + .05}$.

[b]Not significant.

*Significant at the 95% level of probability.

Table 104. Analysis of variance of choice test, 12.64 µ mol DIMBOA/g dry weight diet

Source of Variation	d.f.	S.S.[a]	M.S.	F
Reps	4	0.08	0.02	-[b]
Run	2	0.07	0.035	-[b]
Error (a)	8	0.50	0.06	
Level	1	2.11	2.11	9.59**
Level x Run	2	0.19	0.09	-[b]
Error (b)	12	2.69	0.22	
Total	29	5.45		

[a]Larval counts transformed to $\sqrt{X + .05}$.

[b]Not significant.

**Significant at the 99% level of probability.

Table 105. Analysis of variance of choice test, 25.28 μ mol DIMBOA/g dry weight diet

Source of Variation	d.f.	S.S.[a]	M.S.	F
Reps	4	0.06	0.015	-[b]
Run	2	0.35	0.18	11.25**
Error (a)	8	0.13	0.016	
Level	1	3.75	3.75	15.00**
Level x Run	2	0.17	0.09	-[b]
Error (b)	12	2.97	0.25	
Total	29	7.43		

[a]Larval counts transformed to $\sqrt{X + .05}$.

[b]Not significant.

**Significant at the 99% level of probability.

Table 106. Choice test with DIMBOA treated WF9 leaf discs[a]

Run	Location			
	Replicate	Control	Treated	Dish Surface
I	1	5	3	2
	2	4	3	3
	3	7	3	0
	4	9	1	0
	5	8	2	0
	6	1	5	4
	7	7	2	0
	8	3	7	0
	9	2	7	0
	10	9	0	1
	Sum	55	33	10
II	1	6	1	2
	2	1	3	0
	3	5	5	0
	4	6	2	1

[a]Data recorded are larval counts.

Table 106 (Continued)

Run	Location			
	Replicate	Control	Treated	Dish Surface
II (Cont.)	5	3	6	1
	6	7	1	2
	7	2	1	1
	8	7	2	1
	9	10	0	0
	10	5	5	0
	Sum	52	26	8
	Total	107	59	18
	Mean	53.50	29.50	9.00
	F-value[b]	5.26*		

[b]Split-plot analysis; whole plot = runs; split-plot = treated or control; values transformed to $\sqrt{X + .5}$.

*Significant at the 95% level of probability.